A CLINICAL TELEVISION EVALUATION OF PLAQUE FORMATION IN CHILDREN

By

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Abstract

INTRODUCTION

It has been postulated that plaque acts as an etiologic agent for oral diseases because it concentrates large numbers of microorganisms, localizes them to a specific area, and protects products of bacterial activity from being readily diluted or dissipated. Attempts to associate dental and periodontal disease have been directed to the amount of plaque present.

Our clinical experiences and the epidemiological surveys of periodontal disease provide ample evidence of the fact that we are dealing with a complex disease entity involving the interplay of many etiologic and defense factors. The purpose of this study is to evaluate one of the factors, the rate of plaque reformation in children, and its relationship to their periodontal and caries indices.

REVIEW OF THE LITERATURE

THE INTEGUMENTS OF THE ENAMEL SURFACE OF TEETH

Enamel, the most highly mineralized tissue of the human body, is in very intimate contact with a number of organic substances which exert some influence on it. The tooth is surrounded in the oral cavity by various organic substances, including a number of organic films and saliva, which is also high in organic constituents. The organic substances within, on, or surrounding the enamel and paradontal structures seem to play a dual role; as a protective mechanism in defense against enamel dissolution and caries attack,[1] and as a major factor in the formation of dental plaque, whose relationships with dental caries and periodontal disease will be discussed at length.

Before the formation of dental plaque is discussed, an effort will be made to clarify the confusion over terminology applied to those organic substances. The various terms that have been proposed will be mentioned, and those to be used in this thesis will be identified.

The integuments of the enamel surface may be divided into two major groups: those of embryological origin, i.e., those acquired before tooth eruption, and those acquired only after eruption.

Structures of Embryological Origin

Nasmyth[2] in 1839 is generally credited with first describing the structures of embryological origin which cover the enamel surface before emergence into the oral cavity. He labeled this structure the "persistent dental capsule" which can be separated into two distinct layers.

The inner or acellular layer has been studied and labeled by several authors. In 1921 Gottlieb called it the "primary enamel cuticle" and made the widely accepted statement that this is the final product of the ameloblasts. Other terms assigned to this structure are: "enamel capsule" by Hodson;[3] "enamel cuticle" by Ussing;[4] and "Nasmyth's membrane", after its founder, by Wertheimer and Fullmer.[5] Gottlieb's term, the "primary enamel cuticle", is generally accepted and will be used here. It can be described as being 200-1000 angstroms thick, not birefringent, acidophilic, and stains lightly with eosin.[6] Apparently organic in matter, it is continuous with the prism sheaths of the mature enamel. After emergence into the oral cavity, this structure is quickly abraded by contact and by the abrasive action of foods.[7,8,9]

The cellular layer is generally thought to be formed by the union of the ameloblasts, the stratum intermedium, and the outer enamel epithelium.[9] The cellular layer has been the object of numerous studies,

and the recipient of as many names. Some terms offered in the literature are "united enamel epithelium" by Kronfield[10] in 1943; Orban's[9] "reduced enamel epithelium" the following year; "epithelial attachment cells" by Ussing[4] in 1955; "dental cuticle" and "reduced dental epithelium" by Wertheimer[5] and Sicher,[11] respectively, as late as 1962.

Since the cellular layer is composed entirely of cells, the term "cuticle" seems inappropriate. Furthermore, Ten Cate[12] in 1962 demonstrated that the cellular layers can be distinguished histochemically, which called attention to an inaccuracy in the previously accepted term "united enamel epithelium". The term "reduced enamel epithelium" has been widely adopted and will be used in this review, as it emphasizes the cellular nature of the layer and provides information about its origin and distribution.

Turner[6] in 1958, and other investigators,[12] showed that the "reduced enamel epithelium" is retained for at least a short time attached to the enamel surface after eruption, but is soon removed by the trauma of mastication.[13] Glickman feels that remnants of this structure may be seen in children, appearing as a thin, transparent, colorless surface layer at the cervical portion of the tooth.

Structures Acquired After Eruption

Based on their appearance under the electron microscope, Meckel[14] in 1965 suggested a classification of the various types of organic deposits which could be found, alone or in combination, on and within natural enamel surfaces. These organic deposits originate after the tooth has emerged into the oral cavity. He described a subsurface, a surface cuticle, a stained pellicle, and plaque.

With the aid of demineralized sections less than 1 micron thick and the electron microscope, the fibrillar character of an organic film can be detected within the outermost layer of slightly damaged enamel. Meckel[15] calls this film a "subsurface cuticle." The same author, using enamel chips worn in partial dentures, showed that such subsurface cuticles did not form on absolutely sound enamel, but formed on enamel damaged by very dilute acid.[8] Frank and Brendel[16] in 1966 also reported the existence of an organic matrix in micro-areas where the apatite crystals had been destroyed, and the enlarged spaces between crystals become filled with organic material, probably from saliva.[8]

The Acquired Pellicle

The acquired pellicle, as defined by Leach and

Saxton,[17] is that structure which is acellular and essentially bacteria-free which is only deposited after eruption of the tooth and the loss of the integuments of embryological origin. This structure has probably caused much confusion, as evidenced by its many synonyms. Kirk[18] in 1910 labeled it a "mucin plaque;" "film" was used by Dobbs[19] in 1932; Vallotton[20] in 1945 reported on a "brown pellicle;" Rushton[21] in 1954 named it the "acquired enamel cuticle;" Winkler and Bacher Dirks[7] in 1958, in an attempt to simplify the matter, called all cuticles "dental plaque;" "post-eruption cuticle" was used by Turner[6] in 1958; and Mullin and Smith[22] used the term "dental plaque" to describe the structureless, bacteria-free deposits on dentures.

The term "acquired pellicle" will be used in this paper for this organic layer, as it indicates its post eruptive origin and distinguishes it from the primary enamel cuticles of embryological origin.

The question may be raised as to how the primary enamel cuticle can be differentiated from the acquired pellicle as they both appear structureless, transparent, and essentially colorless on curosry examination. In a 1958 study on both newly erupted extracted teeth and teeth extracted 10 years after eruption, Turner[31] found

several differentiating factors. He found that the primary enamel cuticle refracts light, displays different solubilities in acids, and differs histochemically from the acquired pellicle.

A number of investigators studying tooth surface deposits reported the nearly constant presence of an acquired pellicle.[23,16,24] This is true even on surfaces which undergo attrition.[25,14]

The bacteria-free pellicle is constantly reformed after its removal with abrasives and acts as a diffusion barrier, slowing down the exchange of ions from and into the enamel surface. This same structure serves as the precursor of mature plaque. This will be discussed during the consideration of plaque formation, but it should be visualized at this point that the acquired pellicle is found between the enamel surface and the plaque.[8,26,27,16]

The acquired pellicle appears to follow the contours of the individual bacteria of the plaque at its outer boundary and to develop a progressively more dendritic structure in the direction of the enamel surface.[17] Bodecker[28] stated in 1954 that the most important aspect of the acquired pellicle is its close continuity with the lamellae of the enamel surface which may offer an explanation for this layer's tenacity

to the enamel surface. Manly,[29] in a study of the action of abrasives in dentifrices, found that the acquired pellicle could not be removed by the brush alone and that abrasives were required.

There has been much discussion as to whether the stained pellicle should be listed as a separate entity from the acquired pellicle. For the purpose of this review it will not be, as it is difficult to determine if it is not just a thicker accumulation of the acquired pellicle. Meckel[14] in 1965 speculated that the stained pellicle may be dental plaque becoming structureless due to lysis of its bacteria. In the mouth, it appears as a brownish stained, smooth, and structureless deposit. Vallotton[30] and Turner[31] reported that anywhere from a few days to two weeks was required for its formation. Manly[29] observed the stained pellicle when a dentifrice not containing abrasives was used. Muhler, Dudding, and Stookey[32] found that following a prophylaxis with zirconium silicate and patient's brushing without a dentifrice, that 31 days was required for its reformation.

Dental Plaque

It is very difficult to offer a precise description of plaque because it is a variable and dynamic entity. A general description would consider plaque as an

acquired gel-like mat, closely adherent to a tooth or restoration surface.[33] The mat is composed of an organic film, i.e., the acquired pellicle, microbial masses and their products, organic and inorganic components from oral secretions, shed epithelial cells, and blood cells. The major components are the pellicle, microbial masses, and an intermicrobial matrix.

J. Leon Williams[34] introduced the concept of the thick, closely adhering felt-like mass utilizing photomicrographs in 1897. In a discussion of Williams' paper the following year, G. V. Black[35] offered the term "dental plaque" to describe the mass of bacteria. That term will be used in this review. In 1931 Bibby[35] named it the "muco-bacterial film" and Goldman[36] in 1935 chose the term "materia alba," which has created some confusion.

Materia Alba

Dental plaque should be differentiated from materia alba and food debris. Plaque has a definite architecture discernible with the light or electron microscope. In its mature form at least, it is mainly a product of microbial growth.[23] Krasse[37] based his distinction on the fact that it is so closely adherent to the tooth or restoration surface that it is not

removed by rinsing or a moderate spray.

Materia alba, as pointed out by Muhleman and Schroeder,[38] does not usually exhibit any particular structure even though it is composed in large part of bacteria. As opposed to a product of growth, such as plaque, materia alba is a product of accumulation, composed of microbial masses, desquamated epithelial cells, and blood cells which adhere loosely to surfaces of dental plaques, teeth, restorations, or gingiva. Materia alba can be removed by water sprays or even forceful rinsing.

Food debris, unless impacted interproximally, is readily dislodged by lip and tongue movements, rinsing or gentle streams of water.

The Mechanism of Plaque Formation

An understanding of the mechanism of plaque formation required a correlation of direct visual examination, histological study, chemical assay, and techniques still undeveloped or at least not as yet applied to the problem. In other words, only part of the story is known; and the part that we know is of very recent origin.

According to McDougall,[39] the process of plaque formation may be divided into two stages:

I. The formation of an initial, non-bacterial acquired pellicle, i.e., the development of an "immature type" plaque.

II. The proliferation of microorganisms within the acquired pellicle, i.e., the process of plaque maturation with the final development of a "mature type" plaque.

Development of an "Immature Type" Plaque, i.e., Acquired Pellicle

The staining reactions of the acquired pellicle suggest that it is composed of modified mucoproteins or glycoproteins from saliva, together with lipid material. The specific composition and the actual mechanism of formation remain to be established.

Salivary mucoid, by virtue of its physical properties, will cover every surface in the mouth. As such, it will act as a lubricant in mastication and protect mucous membranes against acid beverages. However, the formation of such a thin mono-layer on tooth surfaces is also the beginning of plaque formation.[16]

This thin, homogenous, transparent, bacteria-free, structureless, acquired pellicle has been studied by many investigators.[23,39,14] Basically, there seems to be general agreement on its physical description regardless of the method used for its study. The common inconsistency by the authors comes about in describing its thickness. Meckel[40] in 1961 described it as two to ten microns thick. In 1963 McDougall[39] found that the

layer varied in thickness from one to fifteen microns, and was of fairly constant thickness over most of the tooth. In a study using plastic strips in 1966, Mandel[25] said that the thickness varied from .05 to .4 microns. Leach and Saxton[17] found a fairly constant pattern in the thickness according to the surface being studied. They found that the interproximal pellicles averaged 3 microns in thickness, 4 microns labially, and an average of 5 microns on the lingual surface. As can be easily appreciated from the above, since the acquired pellicle is thin and transparent, it is extremely difficult to visualize unless it becomes a stained pellicle, as described previously. Basic fuchsin stains the acquired pellicle a light purplish red.[23,8]

McDougall[8] considered the acquired pellicle to be largely salivary mucoids altered by chemical and enzymatic processes, and this is supported by most other investigators. The critical questions of course are: exactly what is this material and how does it come to adhere and build up on the surfaces of the teeth? Few subjects have raised as much controversy. There are almost as many theories as there are authors on the subject; however, a review of these theories may add some insight into the complexity of plaque formation

as a whole.

The idea that salivary mucins are involved in plaque adhesion to teeth seems to have originated with Kirk,[18] who suggested in 1910 that plaques were formed through precipitation of mucin by the action of lactic acid producing bacteria.

Dobbs[19] in 1932 claimed that plaques arise as a result of mucous accumulation on teeth, especially during sleep. Denaturation of the mucoid material by bacterial activity transforms it into a firm resistant plaque by causing precipitation by dehydration.

In 1938 Gore[41] suggested that the carbohydrate portion of the mucoid molecule could be broken down to give acid which in turn would precipitate further mucoid. Gore[42] later published a report in which he admitted that it was very debatable whether such a breakdown of salivary mucoids would reduce the pH sufficiently to cause acid precipitation of mucoids.

In 1958 Winkler and Dirks[7,26] stated their partial agreement with Dobbs in his theory of acid precipitation of mucoid with denaturation of protein by drying with loss of water of hydration. Saliva contains varying quantities, from .05 to .6 per cent, of mucoid.[43] A salivary mucin molecule contains a protein and a mucopolysaccharide moiety.[44] Proteins in solution tend to

spread at a surface. The interaction with the surface changes the configuration of the molecule by surface denaturation to such an extent that the protein coat is difficult to remove. Winkler and Dirks[7] added that the surface denaturation was preceded by adsorption of the protein moiety directly onto the hydroxyapatite of enamel. Ericson[45] recently added some support to this when he found that glycoproteins in saliva, particularly those rich in sialic acid, are most strongly adsorbed to synthetic hydroxyapatite. Pearce[46] in 1966 found that lowering the pH and increasing the calcium ion concentration increased the rate at which proteins are adsorbed onto powdered enamel.

The role of acid production in the precipitation of protein is stressed in many theories; however, Dawes[47] in 1963 and 1964[48] submitted some contrary evidence. He found that parotid saliva would not precititate until a pH of 2.9 to 3.3 was reached: A pH of below 3.5 was required for submandibular saliva. Plaque microorganisms are not known to reduce their environmental pH below 4.0. Therefore, he concluded that acid precipitation of salivary proteins is not likely to be a factor in pellicle formation.

Trester and Kleinberg[49] in 1962, in spectrophotometric studies, revealed that when saliva or an alkaline

solution of plaque proteins is acidified, protein may precipitate even when pH values are only slightly reduced and are still within the physiological range. They also reported that when saliva was incubated, precipitation of salivary protein occurred even at pH levels above seven. They concluded that acid formation aided spontaneous precipitation of protein, but agreed with Dawes, who cast doubt on the importance of acid as a primary effect, but confirmed that acid might favor other processes.

Dawes[50,48] also found in 1964 that adding calcium ion in amounts sufficient to raise the calcium concentration in submandibular saliva by as little as 4 mg. per cent caused precipitation of protein at pH levels as high as 8.6. He speculated that the calcium ion in food or in the exudate from the gingival sulcus might be a factor in plaque formation.

Silverman, Kay, and Kleinberg[51] in 1965 added support to the possible role of the calcium ion. They found that calcium could bind glycoprotein to teeth, besides acting as a binder between bacterial cells.

In 1963 Leach[52,53,17] was the first to observe that oral bacteria removed and used sialic acid from mucin. He suggested that this might lead to formation of a degraded mucin which could precipitate in the

plaque at a mildly acid or neutral pH.

Middleton[54] in 1964 revealed that plaque contains little or no fucose and salivary glycoprotein contains only fucose. This supports the possibility that bacterial alterations of salivary glycoproteins alter their solubilities, resulting in adhesion to teeth and initiation of plaque formation.

McGoughey and Stowell[55] in 1967 found that removing sialic acid and fucose from salivary glycoprotein brought about an increase in viscosity and precipitation. However, they felt that this might not occur until after the mucoprotein had already bound to the enamel apatite, which readily occurs, as shown by Ericson[45] and Pearce.[46] McGoughey and Stowell[55] suggested that the removal of sialic acid and fucose might be a secondary phenomenon.

In 1958 Voreodis and Zander[27] hypothesized that since so many epithelial cells are found in saliva in various degrees of degeneration, these might play a part in cuticle formation. They felt that this was more lokely than the idea that cuticle formation was a product of bacteria.

Another theory was offered in 1966 by Koulourides,[56] who stated that a complexing of the changed organic components might come about with the enamel surface. The polar groups of the altered glycoprotein, i.e., carboxyl, amino acid and hydroxyl, and the surface changes of the enamel could combine.

In all of the various theories presented, there seems to be only one point of common agreement: namely, that cuticle formation results from some type of alterations of salivary glycoproteins. In analyzing the composition of the acquired pellicle, this relationship has been established. In a study of plaque proteins, using starch block electrophonesis, Ferguson[57] reported the presence of numerous proteins with mobilities comparable to those of salivary proteins. McDougall[39] felt that salivary mucoids undergo enzymatic degradation before precipitation, since the pellicle does not give the typical histochemical staining reaction of intact mucoids. Millin and Smith[22] also felt that the pellicle material is salivary in origin, and that the carbohydrate is split off the glycoprotein by the bacteria, leaving a protein core which is difficult to remove.

Armstrong[58] found, in a comparative study of the common amino acids present in the pellicle and in parotid saliva, that the parotid secretion can account for a very small fraction of the pellicle material. The acquired pellicle appears to be derived from submandibular salivary protein. The amino acid moiety of submandibular mucoprotein probably remains unaltered. Armstrong agreed with Leach[52,53] and Middleton[54] that

there is a removal and breakdown of the carbohydrate portion of the molecule before pellicle formation is complete.

Meckel[14] found in 1965 that pellicles formed directly from saliva gave almost identical histochemical staining reactions whether they come from pellicles floated off of freshly extracted teeth, from pellicles formed on enamel segments worn in partial dentures, from pellicles formed in vitro on enamel incubated with saliva, or from dried salivary films.

The role of bacteria in the formation of the acquired pellicle or immature plaque is not well understood and is much debated. As discussed previously, many theories of pellicle formation were based upon changes of pH.[18,7,49] Fermentation of carbohydrate and acid production and resulting protein precipitation were attributed to the bacteria present.[58] Armstrong identified a specific bacterial cell wall component, muramic acid, which indicates the presence of microorganisms in the structure. He suggested that the acquired pellicle may be a complex of salivary mucoproteins and embedded bacterial material.

On the other hand, an in vitro study by Meckel[14] in 1965 showed that inhibition of bacterial growth by antibiotics did not effect the formation of the pellicle.

Meckel stated, "The only essential factor leading to the formation of an artificial pellicle in virto was the presence of saliva." Another observation which cast doubt on the role of bacteria in pellicle initiation was made by Glas and Krasse,[59] who found that a pellicle-like substance formed in germ-free rats.

The immature plaque, i.e., acquired pellicle, will reform after a thorough pumice prophylaxis ranging from 25 hours in most instances[60,22,23] to being present in every section by the third day.[39] This pellicle fills in the minute cracks and lamallae of the enamel surface. Leach and Saxton[17] in the electron microscope study of the acquired pellicle found that it had dendritic processes which appeared to enter the enamel on the proximal surface, and that these processes were lacking on the labial and lingual surfaces. This has been attributed to the presence or lack of damage to the enamel surfaces. They also found that this structure was the same for permanent and deciduous teeth. According to McDougall,[39] the acquired pellicle always appeared on either side of the wedge defects and appeared to spread internally from these defects.

The acquired pellicle seems to form a substrate or platform of adhesion upon which the bacteria develop.[60]

Whether or not such an organic layer for the attachment of bacteria is necessary is not known; however, its presence under plaque seems to be the rule, because the adsorption of mucoproteins on the enamel surface may be faster than the attachment of bacteria.[45] Meckel[8] in 1968 revealed with in vitro tests that bacterial masses adhered much better to an enamel surface by an organic film than on a clean polished enamel surface.

There could be dispute as to whether the acquired pellicle should be regarded as part of the plaque or a separate structure. Frank and Brendel,[16] in their electron microscopical investigation, found that a great number of noncarious mesial and distal surfaces were devoid of any microbial aggregation. In these cases a thin pellicle always covered the most superficial enamel apatite crystals. When a dental plaque is present on a normal or acquired pellicle, it is sometimes easily identified between the enamel apatite crystals and the bacterial layer. However, it is often difficult to locate such a well-defined curicular deposit. In these cases, the cell wall of the bacteria can be in direct contact with the enamel apatite. These observations have also been noted on experimental surfaces worn in the oral cavity to collect plaque.[27,26]

The ultimate fate of the acquired pellicle under established mature plaque or in areas where there has been surface changes in the enamel seems to be unknown. This will be discussed in a description of the inter-microbial matrix.

Development of a "Mature Type" Plaque

The early changes of plaque maturation are characterized by microorganisms occurring on or in close proximity to the acquired pellicle. In the earliest stages the microorganisms generally do not form a continuous layer above the pellicle, but occur as low, dome-shaped, discontinuous clumps. Serial sections show that, in most instances, the thickest portion lies above a wedge defect in the enamel, and it is likely that invasion of the plaque occurs by the growth of organisms remaining in wedge defects after prophylaxis.[39,61] Most of the discontinuous clumps are less than one micron thick and the organisms are arranged with their long axes at right angles to the acquired pellicle. In other areas, a very thin layer of gram positive cocci can be seen covering the acquired pellicle. The morphological types generally occur in separate areas giving the impression that in a given area only one type of organism predominates.

Silverman and Kleinberg[62] in 1967 suggested that bacterial cells, which are colloidal particles in saliva, attach to the acquired pellicle in response to a decrease in pH. They felt that those conditions which favor the deposition of salivary proteins on the teeth, such as a decrease in pH, would also favor the deposition of bacteria from the saliva. With one set of pH on calcium ion levels, the deposition of these cells might be favored over that of the salivary proteins; whereas with another set the reverse might occur.

The subsequent development of the mature plaque depends upon the rapid and progressive invasion of the required pellicle by microorganisms. McDougall[39] found that in 93 per cent of two-day plaque and 34 per cent of one-day plaque there were areas in which almost the entire thickness of the plaque consisted of densely packed microorganisms.

The aggregation appears as the result of adhesive bonds developing between the bacterial cell walls and the acquired pellicle. Adjacent bacteria become adherent through their cell walls or through a matrix that forms progressively and embeds the microorganisms in the plaque.

Frequently, when the organisms reach a height of about 5 microns, a discontinuous zone, one or two microns thick, is seen at the junction of the micro-organisms and the acquired pellicle. This may be due to metabolic activity of the organisms causing dissolution of the immature plaque.

The classical ultrastructural characteristics of the bacteria are conspicuous. The cell walls form the outer envelopes of the bacteria and are built up by more or less complex multilayered sheets.

In retention sites, and in any region where carbohydrates and other nutrients form a suitable pabulum, bacteria will flourish. In fact, microscopical preparation of plaque shows practically nothing but bacteria. These bacteria increase in thickness and numbers while continuing to produce destructive metabolites.[7] The mature dental plaque has an average thickness of approximately 200 microns.[14]

As was mentioned previously, the mature dental plaque consists of the acquired pellicle, the inter microbial matrix, and the microbial mass. A description of the acquired pellicle has been given. The importance of the acquired cuticle in plaque formation is readily appreciated, yet it comprises a small portion of the mature dental plaque. Winkler and Backer Dirks estimated

that dental plaque is approximately 70 per cent bacteria[7] and 30 per cent interbacterial matrix. These remaining two entities of dental plaque will now be discussed.

Frank and Brendel[16] described great microscopic variations in the character of the interbacterial matrix. In some areas, the microorganisms are closely packed and almost no interbacterial matrix is present filled by a non-dense material. Sometimes large interbacterial spaces are present which appear to be empty. They feel that this is an artifact. Finally, broad interbacterial spaces can be filled by a dense matrix in which some very fine filaments on some granular material can occasionally be seen.

Precipitated salivary mucins have been believed to form the plaque matrix.[63,18,17] Frequently, the interbacterial matrix separating the microorganisms of the plaque has a similar ultrastructure, and is continuous with the acquired pellicle located between the superficial enamel apatite crystals and the inner layers of bacteria of the plaque. In other cases, the interbacterial matrix is adjacent to the acquired pellicle which is of a greater electron density as viewed with the electron microscope.

Dawes and Jenkins[47] in 1963 felt that the matrix is formed by the precipitation of salivary mucoids. In contrast to mucoids, however, plaque was found to contain no sialic acid. These authors offered the explanation that bacteria have the ability to metabolize sialate. The findings suggest that the protein matrix of plaque may be a partially degraded form of salivary mucoid from which sialate has been removed.

Trester and Kleinberg[49] in 1962 found that the nitrogenous material of saliva, i.e., mucoprotein, precipitates spontaneously to form plaque matrix, with the pH of the local areas largely determining the rate of precipitation.

McDougall[39] has cast some doubts on the assumption that salivary proteins are the main source of plaque matrix. He feels that the acquired pellicle was formed from altered salivary mucoids, but as the bacteria invaded it, "they seemed to have caused its complete removal."

Recently, however, the idea that the interbacterial matrix consists of extracellular bacterial polysaccharides is favored by many.[64,65,66,67,68]

Wood and Critchley[66] in 1967 found that certain microorganisms are capable of producing the extracellular polymers, dextran and levon, from sucrose.

These polymers contribute the gelatinous or gummy characteristic of plaque[60] and contribute bulk to the plaque as well as providing a sticky medium to which bacteria can adhere.[69]

The original tendency to attribute the property of extracellular polysaccharide production to one specific type of streptococcus[65,70] has been modified to include filamentous lactobacillus[71] and many other types of organisms.[72]

Gibbons and Banghart[65] found several characteristics of the extracellular dextran which could be important in enabling it to function as interbacterial matrix: it is able to adhere to powdered hydroxyapatite or apatite which has been coated with saliva; it is able to form an insoluble complex when incubated with saliva; and it is relatively resistant to hydrolysis by mixed bacterial growth from samples and saliva, which means it is biologically stable in the mouth and thus well suited to function as a matrix for plaque.

In 1967 Wood[73] studied 24-hour plaque growths. He concluded that the extracellular polysaccharides, dextran and levan, comprise approximately 9 per cent of the dry weight of plaque. He concluded that since the matrix consists of about 30 per cent of dental plaque and the polysaccharides nearly 10 per cent of

the total dry weight, the polysaccharide constitutes approximately one-third of the plaque matrix.

Presently, one of the most exciting aspects of dental research is the investigation being conducted into the bacterial composition of dental plaque. All of the cultivable bacteria present in high dilutions of dental plaques were isolated and their relative proportions determined by Gibbons et al[74] in 1964. He estimated that microscopic counts average 2.5×10^{11} bacteria per gram. Gibbons and Socransky[75] took plaque from five individuals and found that a total of 361 bacterial strains were isolated. A classification of composition according to percentage of total volume revealed that gram positive facultative cocci were the single most numerous group of organisms isolated. They average 28 per cent of the cultivable microbiota of plaque, but because the organism tends to form aggregates, this estimate may be low. The composition of the remaining organisms is as follows: gram positive facultative rods, 24 per cent; gram positive anaerobic rods, 18 per cent; anaerobic gram positive cocci, 13 per cent; gram negative anaerobic rods, 10 per cent; gram negative anaerobic cocci, 6 per cent; and the filamentous organisms which comprise about 4 per cent of the bacterial population make up the

majority of the bulk on a volume basis. It should not be inferred that this is a static ratio or that this ratio will exist from person to person.

The changes in the bacterial population's ratio is exemplified and explained in a study conducted by Ritz[76] in 1967. He used fluorescent antinocardia serum to stain cross sections of one and three-day-old plaque to study microbial population shifts in plaque. He found in one-day plaques from 30 to 100 microns thick that fluoresced nocardia was a relatively large proportion of the total plaque population. However, in the three-day-old plaques ranging from 100 to 200 microns thick, the proportion of nocardia had decreased and the fluoresced cells that were present were concentrated on the outer surfaces of the plaque. The explanation offered suggests that aerobic bacterial growth first occurs at the tooth surface until the plaque is thick enough for anaerobic conditions to prevail in the deeper layers suitable for growth of the strictly anaerobic organisms, such as fusobacteria, veillonella, and spirochites. For longer periods up to nine days, levels of the microaerophilic and facultative, i.e., actinomycies and corynebacteria, and of the anaerobic groups, i.e., veillonellae and fusobacteria, had increased. These population shifts are believed to be

due, at least in part, to the nature of the plaque environment with respect to levels of exygen present, i.e., the oxidation-reduction potential. Gibbons et al[74] estimate that the number of bacteria cultivated anaerobically is approximately 4.6×10^{10} bacteria per gram as compared to 2.5×10^{10} bacteria per gram cultured aerobically.

Mandel et al[77] in 1957 and others[78,79] have noted that preponderance of coccoid forms rather than filaments in the early plaque material. Howell, Rizzo, and Paul[80] noted that the filamentous organism increased from 4 to 10 per cent in from 14 to 21 days. Ennever, Robinson and Kitchen,[81] in describing 30-hour plaque, observed filamentous bacteria, roughly parallel to each other and approximately at right angles to the point of attachment. They also ntoed clumps of other bacterial forms which appeared to be supported by the filaments. Dietz[21] suggested the hypothesis that the filamentous organisms which are in plexiform arrangements offer at least mechanical retention for smaller bacteria.

McDougall[24] in 1963 noted that many filamentous microorganisms terminated on, or in, the acquired pellicle; he felt that the ends of the filaments were embedded firmly in the pellicle, as this would help explain the

firm attachment of the plaque to the tooth surface. The penetration of these organisms into lamellae, wedge, and other defects undoubtedly also contributed to this firm union. Jay and Voorhees[82] also raised the question whether these interwoven threads do not contribute materially to the cohesiveness of the whole mass.

Studies on Plaque Formation

As will become very apparent in a review of past investigations into the complexities of plaque formation, plaque has only within the past ten years begun to receive the attention it warrants. In the past, three general methods have been applied to the quantification and qualification of plaque or calculus deposition per unit of time; assessment of the areas covered by the deposit either by subjective estimation, direct measurement on the tooth or indirect measurement from enlarged photographs; histologic evaluation of deposits removed from extracted teeth on surfaces placed in the oral cavity to simulate the enamel surface; gravimetric procedures involving the material removed directly from the tooth surfaces or using "standardized foils" as the intermediary.[25]

J. Leon Williams[34,83] presented a paper to the New York State Dental Society in 1897 in which through

the use of photomicrographs he became the first to demonstrate masses of microorganisms attached to the tooth surface in beginning caries or enamel. He put forth the concept that covering the surface where decay had commenced there was always a thick felt-like mass of acid-forming microorganisms.

In 1940, while demonstrating the effectiveness of tooth brushing, Hank[84] noted that a thin, fairly tenacious growth of plaque can be demonstrated on teeth in most mouths from 24 to 36 hours after all the plaque has been removed by instrumentation. While studying the relationship between plaque and caries, Frisbie and Nickolls[85] in 1947 observed plaque formation on plastic cover glasses attached to oral appliances.

Appleman, Freese, and Riera[78] in an effort to histologically analyze plaque formation in 1955 used detachable plastic slides attached to the buccal surfaces of orthodontic bands.

In 1957 Mandel et al[77] gave the first report on a new method to study calculus formation. The method used celluloid strips adapted tothe lingual surfaces of mandibular anterior teeth and held in place with ligature wire. These strips were removed at different times, so that sequences of formation could be

histologically examined.

Winkler and Dirks[7] in 1958 stressed that plaque formation is essentially a local process. Plaques will be thick in regions where acid production is favored by food retention and thin over smooth surfaces which are cleaned naturally. They hypothesized that the anatomical forms of various surfaces and the width of interdental spaces will also influence the size and the properties of the plaque.

In 1960 Turesky, Renstrup, and Glickman[86] used sections of cellulose acetate crowns ligated to the lingual surfaces of mandibular incisors on a group aged 5 to 70 years to study early calculus formation in children and adults. No brushing was allowed in from one to 30 days. The authors found that plaque accumulated early in the study and that it accumulated in greater amounts in those celluloid crowns which were roughened before insertion. This point was confirmed by Brebau and Muhleman.[87] Also, the authors found that the amounts of accumulation varied in different mouths and in different strips in the same mouth. Manthalen, Schoeder, and Muhleman[88] evaluated the reproducibility of foils adapted to lower incisors and found 16 per cent error.

Green and Vermillion[89] in 1960 suggested a method of making objective estimates of the amount of plaque covering the surfaces involved. This is done by running the outside of a number five explorer along the buccal or lingual surfaces and noting the occlusal and incisal extent of the debris as it is removed from the tooth surface.

In 1963 Stout, Swanson, and Mott[90] devised a method which can be used for in vitro studies. Using a custom-designed measuring flask, they employed the principle of fluid displacement to measure volumes of irregular objects. First they measured the amount of fluid displacement caused by the teeth, and then by the teeth plus their accumulations. The volume of the accumulations in each instance was the difference between the two readings. This method was tedious and time-consuming; however, duplicate readings were obtained within $\pm$ .02 cc.

McDougall[39] in 1963 made a histological and histochemical examination of extracted teeth at intervals from 0 to 14 days following a dental prophylaxis. He found that once the bacteria invaded the acquired pellicle, which generally took one to three days, there was a rapid and progressive invasion and replacement of the homogenous non-bacterial matrix by the microbial

masses. He also found that early plaque forms with diets containing no sucrose and in some subjects after 18 hours of fasting.

Sumter Arnim[91] in 1963 studied the rate of plaque formation using disclosing tablets, color transparencies, tracings, and a planimeter to measure the area of the plaque involvement. He found that seven days growth of microcosms with no personal hygiene of the subjects covered from 43 to 65 per cent of the labial surface of the mandibular and maxillary incisors. He also found that fibrous foods cleaned from 3 to 19 per cent of the labial surfaces of anterior teeth. Arnim used a planimeter to make his measurements and described the process as accurate but laborious. The planimeter is used by guiding it around the periphery of the tracing of the tooth surface five times in one direction and then reversing it and tracing the outline five times in the other direction. The average of the 10 recorded measurements is considered to be the area of the surface.

Kleinberg and Jenkins[92] observed plaque formation and plaque pH in 1964, by studying patients who did not brush for three days and who had no preceding prophylaxis. They found that the incidence of periodontal disease decreased from posterior to anterior in the

maxilla, with the reverse being true in the mandible. These findings were consistant with the plaque pH patterns.

In 1964 Silness and Loe[70] expressed the view that for the visual assessment of the amount of plaque present, moving an explorer along the surface of the teeth both supragingivally and subgingivally gave better results than disclosing tablets. They also found that 70 per cent of the total amount of plaque on the teeth was interproximal, 20 per cent lingual, and 10 per cent labial. They observed no major differences in the tendency for plaque formation between maxillary and mandibular teeth or between different groups of teeth.

Bjorn and Carlsson[93] in 1964 studied dental plaque morphogenesis directly on tooth surfaces by examining with a stereomicroscope plaques visualized with disclosing solution. Teeth were cleaned to the point of no disclosable plaque and the patients were asked to refrain from brushing. McDougall[39] and Bagdale, Bahn and Modovia[94] and Remeskis, Gerloch, and Englander[61] found, however, that a dental prophylaxis did not remove bacteria from the grooves and fissures of the enamel surface. Facial surfaces of anterior

teeth were examined every day for a week. At the end of the first day, McDougall noted not only light staining material which was almost free of micro-organisms. From two to four days, the surface coating stained intensely and filled up cracks and defects on the enamel surface. In a number of areas, especially along the gingival margin, there appeared discrete, small hemispherical, intensely staining plaque colonies, which when examined microscopically were essentially coccoid microorganisms. At five days, the plaque colonies were covered by haloes. After five days, it was noted that the haloes increased in stainability, and grew and became confluent with their neighbors; that plaque colonies increased in size; and that with an increase in thickness the plaque lost any further morphological characteristics. Bjorn and Carllson[93] also ran control studies which proved that basic fuchsin stain did not influence the morphological pattern of plaque formation, but did retard its growth.

Carlsson and Egelberg[70] used basic fuchsin stain, color transparencies, and oriented compound bites to study the effects that high protein and various carbohydrate diets had on plaque rate and morphogenesis. They found that when a high protein diet was supplemented every half hour with sucrose rather than glucose

or fructose, the rate of plaque formation was much faster with the sucrose supplement as well as differences in morphology. They observed that in three days pellicle formation was absent and that a voluminous, turgid plaque which did not cover the entire surface had formed. With the other carbohydrates, on strictly a high protein diet, pellicle formation was observed, with a thin, smooth plaque covering the entire surface formed in three days.

Meckel,[14] using an electron microscope in 1965, found all the surface cuticles, i.e., subsurface, acquired pellicle and dental plaque, when he embedded pieces of enamel in removable partial dentures. He did not find any stained pellicle in this group as the materials were worn for only 10 days; however, in another experiment in which they were worn for 215 days, a stained pellicle was found.

Poole and Gilmour[95] in 1965 studied naturally occurring plaque and plaque accumulated on a removable membrane. They found no differences at the five per cent level. These observations were based on the differential microbial population and the number of organisms per milligram of nitrogen.

In 1965 Rayes and Smith[96] compared the effectiveness of hand versus motor driven toothbrushes in the

removal of plaque. They used the same basic technique as Arnim, but took the photographs at a fixed distance by using a cephalometric head holder. They felt that this method of using photography and the planimeter was accurate and reproducible.

Also in 1965, Slack and Bowden[79] reported on an experimental plaque device which was positioned in vivo against a non-carious enamel surface. The device consisted of a welded orthodontic stainless steel band with a removable part in which a short length of a soft polyethylene tube was inserted to carry the medium on the surface where the plaque is to grow. They confirmed that the coccal forms are first established, after 5 to 7 days the filamentous forms are in evidence, and by 14 days the filaments seem to predominate.

Egelberg[97] in 1965 studied the effect of soft and hard diets on plaque formation in dogs. The results indicate that dogs accumulate more bacterial plaque when given a soft diet than when given a hard diet. He felt that the difference was mainly due to the mechanical cleansing of the hard diet. He used basic fuchsin, and took standardized color photographs by impressions on bite plates in compound connected to the camera with a fixation arm.

Carlsson and Egelberg[98] in 1965 with a continuation of the above study, found that adding sucrose to both the soft and hard diets did not influence the amount of plaque formed. They said this finding might be due to differences of the oral flora in man and dogs.

Kinoshita[99] used .1 per cent basic fuchsin to stain the labial surfaces of lower incisors and color transparencies in 1966, to evaluate the rate of plaque formation. The transparencies were projected at 65 times magnification, and the stained plaque and unstained tooth surface areas were traced on paper and subsequently cut out for gravimetric determination. The percentage of stained tooth surface in relation to the total tooth surface was used as a measure of the extent and rate of formation. He found that on the average that 12 per cent of the labial surface was covered after three days. He also observed no significant differences between the toffee, gum, and control group.

In 1967 Kaminsky and Kleinberg,[100] in a study to evaluate changes in the inorganic composition of plaque as a function of time, noted a pattern which was identical to the pH pattern observed previously. They found that the calcium, phosphorous, and ash levels in all areas were highest at one day, decreased sharply at two days and either remained unchanged or rose or

fell slightly by the third and fourth day. There was a lower percentage of ash in mandibular plaque than in maxillary plaque. This was attributed to the proximity to submaxillary saliva, and this was also given as the reason for the higher pH level for the same area. The calcium, phosphorus, and ash to dry weight of plaque were least for maxillary labial plaques, most for mandibular interproximal plaques, and intermediate for maxillary interproximal and mandibular labial plaques.

Chatterjee and Kleinberg[101] also investigated the carbohydrate level of plaques of different ages. They found that carbohydrate levels for all areas increased markedly in the second day and fell to first day levels on the fourth day. This pattern was just the reverse for the calcium, phosphorus and ash levels. The explanation offered for these findings was that the cellular portion of plaque has an increased carbohydrate and decreased calcium and phosphorus levels, as compared to the acellular portion. One-day plaque contains a higher cellular ratio than does two-day plaque.

Ratcliff[83] in 1968 studied 68 males between 18 and 45 years of age. A prophylaxis was given and teeth were stained before and after meals for a 72-hour period. He found the largest increases occurred during the night,

with only slight increases between breakfast and the evening meal. He indicated similarities of accumulation patterns between the six maxillary anterior teeth and the six mandibular anterior teeth.

Hutchins et al[102] in 1968 used methylene blue stain to visualize the outline of the plaque, and a laminated plastic measuring head forming a 3/16 inch equilateral triangle attached to a stainless steel wire handle to measure the plaque. Measurements were made from the free gingival margin to the highest level of uninterrupted stainable material. This was attempted to take subjectivity out of visual estimates of plaque accumulation. They found that 16 examiners were able to reproduce each other's measurements within five per cent.

In 1968 Bibby[60] emphasized, as do many of the studies reviewed, that plaque is dissimilar from one individual to another and also from area to area on the same tooth. Structural dissimilarities may be the result of biochemically different experiences on different areas of the tooth surface. According to their location, bacterial composition or thickness, the biochemical activities taking place in them will not be the same at different depths in the same plaque.

Pathologic Potential

Plaque acts as an etiologic agent in several oral diseases, mainly because it concentrates large numbers of microorganisms, localizes them to specific areas, and protects products of bacterial activity from being readily diluted or dissipated. The mature plaque is essentially a bacterial community. The metabolic activities of the residents vary with local conditions and can result in the production of many different substances which can markedly affect adjacent tissues.

Kleinberg and Jenkins[18] expressed the opinion that dental plaque might be considered to be acting as an "environmental" membrane between tooth and saliva, with the metabolism of plaque playing a major role in the net movement of calcium salts between these two compartments, i.e., deposition of calcium salts associated with periodontal disease and the removal of calcium salts from the tooth surface with caries.

Frank and Brendel[16] in 1966 stated that plaque can be classified, according to behavior, into two types. In one type, located principally over the cervical enamel regions and possibly on denuded cementum, the dental plaque, undergoing calcification, is transformed into dental calculus without any apparent deleterious effect on the enamel surface. In the

second type of plaque, the bacterial aggregation attacks the enamel surface and is associated with the development of incipient enamel caries.

Since 1944, when Stephan[103] found that the pH minimum levels in plaque on the maxillary anterior teeth were inversely related to the caries rate, the differences between pH levels both locally and in individuals of varying susceptibility have received a great deal of attention.

Kleinberg and Jenkins[92] postulated that plaque pH pattern and the incidence of both caries and periodontal disease are related, and that these conditions generally occur at different pH levels.

In 1946 Keyes,[104] in conducting a study on plaque formation periodontal disease, and dental caries in hamsters found that the extensive accumulation of plaque material and periodontal disease were generally, but not always, associated with dental caries.

Hemmes, Cohen, and Manly[105] in 1964 analyzed plaques from nine caries-active individuals and compared them with the plaques of nine caries-inactive individuals. No difference could be observed in the dry weight per unti volume between the two types of individuals. This study illustrates that the composition of the plaque must be the determining factor as far as

pathogenicity is concerned. Recently, it has been found that certain microorganisms display several qualities of specificity which enable them to be of pathological importance.

Frank and Brendel[16] noted eight vesicles within the cytoplasm of human plaque organisms with the electron microscope. They have been noted in coccoidal and rod-shaped, as well as in filamentous forms, and have been interpreted as stored intracytoplasmis polysaccharides. The authors emphasized that only a few bacteria in human approximal plaques located over normal non-carious enamel showed vesicles of polysaccharides in their cytoplasm, whereas a great number of actively storing polysaccharide organisms have been noted over carious lesions.

Gibbons[106] estimates that 54 per cent of organisms cultivable from plaque are capable of storing large quantities of polysaccharides from caries-active individuals, whereas only 29 per cent of organisms from caries-inactive plaque had this ability.

The fact that plaque of caries-active individuals had a lower testing pH than did plaque of individuals who were caries-free has been noted by many investigators.[107,103] Gibbons and Sacronsky[108] and others offer a possible explanation of this: namely, that it

is due to the production of small amounts of acid over relatively long periods from carbohydrate reserves of the glycogen-amylopectin type, of the plaque microbiota. Organisms store the carbohydrates when exogenous carbohydrate is plentiful and catabolize it when nutrients become sparse, thus keeping the pH low for a long period of time. Streptococcus mitis was observed to store larger quantities of intracellular carbohydrates than any other organism found. When exogenous carbohydrate is not available, streptococcus mitis catabolized stored polysaccharide, and it is converted to lactic acid. Thus, acid produced by plaque microorganisms can maintain plaque at an acid pH. Thus, in the human dental plaque, organisms can lower and maintain the pH of packed organisms to pH of 6 for over two hours in the presence of a continuously flowing buffer. The very low pH attained in the absence of flowing buffer, for example, during sleep when salivation is diminished, may indicate that acid derived from polysaccharide catabolism could be of etiologic significance in the caries process.[109,110,111]

It has also been found that both rodent and human cariogenic streptococci synthesized large quantities of extracellular polysaccharides of the dextran-levan type, particularly from sucrose, whereas strains of non-cariogenic

bacteria formed only trace amounts.[65] Jordan and Keyes[110] developed an in vitro method of studying streptococci known to be caries-conducive in experimental animals. They formed a heavy bacterial plaque on extracted teeth, artificial teeth, and stainless steel wire. Streptococci known to be caries-inactive in animal tests did not form plaque under these conditions. The authors concluded that the ability of specific strains of organisms to colonize and form plaque on the tooth surface in the presence of sucrose and mucin would give them an advantage over the non-plaque-forming strains in the initiation of carious lesions.

Pathologic specificity of certain organisms is attributed to the ability of the plaque at the gingival crovice to produce hyaluronidase, collaginaseses, proteinasis, endotoxins, and other metabolic destructive by-products. These metabolites can destroy tissue directly or at least widen intercellular adhesion and promote the passage of irritant and destructive substrances through the epithelium.[111,112]

Even in 1897, when Williams[83,34] was presenting his photomicrographs, he observed that the dental plaque was a mass so dense and adhesive as to make it highly improbable that the enamel was affected by any

acid other than waht the bacteria were excreting at the very point where they are attached to the enamel. Williams also felt that the mass prevented excreted acid from being washed away, so that it exerted full chemical power upon the calcific or periodontal tissue. The most widely studied plaque activity is the production of acid when glucose, sucrose, and other soluble sugars are introduced as substrate. The resultant fall in pH to the point at which enamel dissolution occurs is considered by some investigators[113,114,115] as the initial step in dental caries. The plaque bacteria also produce chemical entities which can complex calcium at neutral pH's and enzymes which can destroy the mucopolysaccharide ground substance and the structural proteins. Although many specific aspects of the destructive process remain to be understood, the bacterial character of the disease and the direct involvement of the plaque seem well established.[56,39] Frank and Brendel[16] found that a normal non-carious tooth surface can be covered by a microbial aggregation without any deleterious effect on the superficial apatite crystals. On the contrary, in all the carious enamel surfaces studied, the presence of a dental plaque over the diseased enamel was a constant feature and no caries developed in the absence of a superficial

dental plaque.

In 1962 Pomeiger and Manly[116] noted the relationship of plaque to caries clinically or in vivo, but no attempts to prove this relationship in vitro had been made. They then used human plaque in vitro, placing it in contact with enamel sections and immersing it in a sucrose media. They found that plaques one millimeter thick which were treated with sucrose for 30 minutes had the ability to decalcify underlying enamel for a period of time considerably greater than 30 minutes. They suggested that the production of intracellular and extracellular polysaccharide is the mechanism responsible for this phenomenon.

Young, Higuchi, and Koulourides[117] in 1967 put forth the theory that plaque thickness may determine whether demineralization can occur. A thick plaque will generally favor dissolution of the enamel mineral substance, according to the theory. However, if the plaque is sufficiently thin, the buffering action of the saliva overwhelms the mineral dissolving tendency of the plaque and no demineralization occurs.

In 1968 Koulourides[118] attached enamel facets to prosthetic devices. Single versus double layers of gauze, .15 and .37 millimeters, respectively, covered the enamel on the assumption that the thickness was the

controlling factor. He found that doubling the thickness of gauze, i.e., plaque, increased the rate of enamel penetration in most cases.

In a statistical evaluation, James[119] compared dental cleanliness with the DMF of over 2000 11- and 12-years-olds in England. He found that in females with excessive amounts of debris, there was an increase of 23 per cent in the DMF, and an increase of 21 per cent in males. The differences in the DMF increases were significant at the one per cent level. The World Health Organization agreed upon this at a symposium in 1961, stating in its report: "The presence of dental deposits, whether mineralized or not, is undoubtedly the most important factor in the development of periodontal disease."

Bacterial populations in close approximation to mucosal surfaces of the periodontium reach numbers at least as high as 10^{10} organisms per gram of plaque. It is axiomatic that a consideration of their metabolic potential and their capacity to disrupt normal tissue metabolism is essential to an understanding of their metabolism of the periodontium.[111]

There is substantial, but not conclusive, evidence that the flora of dental plaque is responsible for the inflammatory and suppurative phenomena found in

periodontal disease. Support for this theory comes from a variety of sources.

The pathogenicity of organisms, as demonstrated by MacDonald, Gibbons, and Socransky[111] in the laboratory by injection of gingival debris subcutaneously into guinea pigs resulted in the development of abscesses.

An electron microscope study by Listgarten[120] revealed the presence in underlying tissue of crevicular microorganisms in cases of ulcerative necrotizing gingivitis.

The production of certain bacterial metabolites, i.e., hyaluronidase, etc., can destroy tissue or at least widen its intercellular spaces.[111]

Mitchell and Holmes[121] carried out a study on institutionalized patients in which they found that antibiotics, i.e., vancomycin, can reduce gingivitis even without supportive oral hygiene.

Jordan and Keyes[122] demonstrated that periodontal disease can be induced in uninfected hamsters by inoculation of aerobic, gram positive filamentous bacteria from plaques of hamsters in whom periodontal disease had occurred spontaneously.

Loe, Theilade, and Jensen[123] found that periodontal disease could be produced experimentally in man by a

refraining from brushing, which causes an increase in plaque formation. With a return to normal oral hygiene, plaque scores return to normal and gingival inflammation is reversed.

The relationship between the presence of dental plaque and periodontal disease is not easily established from an epidemiological standpoint.

In 1958 Lovedal, Arno, and Waerbaug[124] studied 1400 Norwegian factory workers to evaluate the effect of strictly local treatment of periodontal disease. They concluded that a correlation exists between the efficiency of oral hygiene and the incidence and distribution of subgingival calculus, gingivitis, and pathologically deepened pockets.

Green and Vermillion[89] observed 577 males between 11 and 17 years of age in 1960 and found a correlation coefficient between oral debris and periodontal scores of .6 with a P value of .001.

Green,[125] in another study conducted in 1960 on 1750 males in the same age group in India, found that oral hygiene scores were associated positively with the periodontal disease scores.

O'Leary, Shannon, and Prigmore,[126] in 1962, found a good clinical correlation between plaque and gingivitis. They reported a correlation coefficient of .68

and a P value of .01.

In 1963 Russel[127] reported a good correlation between the Oral Hygiene Index and Russel's Periodontal Index.

In a study of 5,685 people from Equador and Montana in 1962, Green[128] made the generalization that people with good oral hygiene status, regardless of age, have a low Periodontal Index Score. Conversely, people with poor oral hygiene status, regardless of age, have a high Periodontal Index Score.

In a study of individuals in the age group from 12 to 30 years, Chowla, Nanda, and Mathus[129] in 1964 found a relationship between bacterial plaque and gingivitis. They observed that the gingivitis score became higher as the plaque scores increased.

In 1964 Ash, Glitin, and Smith's[130] correlation coefficients between plaque and gingivitis formed from 5 to 7 days after prophylaxis were .285, which was not significant at the one per cent level of confidence. However, a correlation coefficient of .66 was found in the 30 to 60 day group.

There have been several studies conducted in which a positive correlation was not found between plaque and periodontal disease.

In 1950 Massler, Schour, and Chapia[131] conducted an examination of suburban Chicago school children and found no correlation between plaque and gingivitis.

In a study conducted in India in 1961, Ramfjord[132] found a virtual lack of oral hygiene, and that 95 to 100 per cent of all teeth examined had plaque accumulations. Almost 100 per cent of the participants had periodontal disease of one form or another. However, no apparent correlation was found between the gingivitis and plaque scores for the individual teeth. However, no apparent correlation was found between the gingivitis and plaque scores for the individual teeth. However, an indefinite correlation appeared between the gingivitis score and the percentage of teeth with bacterial plaques. He surmised, on the basis of clinical observations, that the gingival part of the coronal plaque is the main source of the gingival irritation and the extent of the rest of the crown that is covered in very extensive plaque formation is apparently of lesser importance.

Hoover and Robinson[133] in 1962 also found a lack of correlation between plaque and gingivitis when studying the effectiveness of automatic and hand tooth brushing.

Silness and Loe,[134] in a study of pregnant women in 1964, did not find related increases in plaque to go along with increases in the gingival index. They felt that other factors were involved.

Generally, calculus formation can be divided into three distinct stages, according to Mandel[77] and Theilade.[26] First is the formation of the acquired pellicle, which is followed by the deposition of dental plaque, and then by the mineralization of the existing dental plaque.

Several investigations have established the association between dental plaque and its constituents and calculus formation: the formation of calculus on plastic strips conducted by Muhleman and Schroeder[38] when studying the dynamics of calculus formation; the in vitro calcification of bacterial plaque and plaque bacteria;[135] and the intraperitoneal calcification of plaque bacteria, as observed by Rizzo, Scott, and Bladen.[136]

Although plaque pH measurements are usually associated with studies of dental caries, the information may also be significant in relation to calculus formation and periodontal diseases. Kleinberg and Jenkins[92] found that just as areas of low pH tended to coincide with regions of higher caries incidence, areas of greater

alkalinity could be related to the distribution of periodontal disease. Regions that showed the greatest tendency for periodontal disease, the interproximal and lingual surfaces, exhibited a greater alkalinity than the buccal surfaces. Similarly, the increase in the intraoral incidence of periodontal disease from anterior to posterior in the maxilla and the reverse direction in the mandible paralleled the relative plaque pH values.

In 1960 Toresky and Renstrup[86] microscopically examined dental plaques of patients from 5 to 70 years of age. They found that there was microscopic evidence of calcification in all specimens between the second and sixteenth day in the 13 to 70 year old group. Theilade[137] made a similar observation.

However, continued Toresky and Renstrup,[86] no calcification occurred during the 30-day period in four out of fourteen patients in the 5 to 12 years old group. In the remainder, calcification was the same as in the older group. They concluded that once the soft plaque accumulates, it undergoes calcification in children in much the same way as in adults, but at a slower rate.

Loe[138] feels that although there is a close relationship between the distribution of calculus and

periodontal disease, it is apparent that periodontal disease is more common than calculus. Microscopical investigation of the relationship of calculus to the gingiva indicates that it is not the mineralized part of the deposit that plays the major role as the gingival irritant. Calculus is probably always covered by an unmineralized bacterial deposit. There seems to be no doubt that the microorganisms which are in this way in direct contact with the epithelial cells of the gingiva initiate and maintain the periodontal inflammation. Thus, it follows that inadequate margins of restorations, as well as untreated carious lesions, act in the same way as calculus, i.e., to provide retention for bacterial plaque.

METHODS AND MATERIALS

A total of 56 children, who were receiving treatment in the Pedodontic Department at Indiana University School of Dentistry, ranging in age from eight through 12 years were observed in this study. The criteria for selection were that both the maxillary and mandibular permanent central and lateral incisors be fully erupted, caries free, and in normal alignment for that age group. Since intraoral photography was used in this study, only these teeth were evaluated.

At the first appointment, each child was examined and both a caries and periodontal index score established. A compound bite mounted on a plexiglass bite plane was taken to orient the Polaroid CU5 camera with an extension so constructed on the lens mount to give a consistent three times magnification of the area of study.

The DMFS index was chosen to relate caries experience of the childs entire mouth, it is briefly described as the following:

1. A definite "break" in the contour of the enamel surface.
2. Loss of the "normal" color of the tooth at the site of the lesion, as evidenced by comparison to non-carious regions of the same tooth.

3. Definite retention of the explorer point in the carious lesion. "Feel" is important here to distinguish between retention from incipient carious lesions and extensively deep, but non-carious fissures.

A total of five bite-wing radiographs were taken on each patient by a dental hygienist. These included two posterior bite-wings and three anterior bite-wing radiographs. All radiolucent areas due to demineralization were scored. A transilluminator was also used to detect anterior lesions.

Other factors were considered for scoring, as follows:

1. When tallying DMFS scores, a restoration in the tooth or the surface took precedence over secondary caries, although both caries and restorations were recorded for each surface.
2. A temporary restoration was scored as a restoration.
3. A full crown was scored as involving all surfaces.
4. Teeth already missing were scored thus when determining missing surfaces: 5 (3) for posterior teeth and 3 (2) for anterior.

(This is in contrast to teeth indicated for extraction during examination; thus all surfaces involved are recorded.)

After the caries were scored, a thorough periodontal examination was performed using a mouth mirror and periodontal probe.[127] The scoring and criteria for Russel's Periodontal Index were utilized and briefly described as follows:

Score 0 Negative. There is neither overt inflammation in the investing tissue nor loss of function due to destruction of supporting tissues.

Score 1 Mild Gingivitis. There is an area of inflammation in the free gingiva which does not circumscribe the tooth.

Score 2 Gingivitis. Inflammation complete circumscribes the tooth, but there is no apparent break in the epithelial attachment.

Score 6 Gingivitis with pocket formation. The epithelial attachment has been broken and there is a pocket. There is no interference with normal masticatory function; the tooth is firm in its socket, and has not drifted.

Score 8 Advanced destruction with loss of masticatory function. The tooth may be loose, may have drifted, may sound dull on percussion with a metallic instrument, may be depressable in its socket.

To arrive at an actual index, for the entire mouth of the child, each tooth is scored according to the above table; the scores are totalled; and the total is divided by the number of teeth present.

The maxillary and mandibular anterior segments were also assessed by P-M-A Index. This method served as a comparison with Russel's Periodontal Index for the age group examined. Each gingival unit in the anterior segment was divided into the papillary, marginal, and attached areas and scored as follows:

Papilla

0 - Normal; no inflammation

1+ - Mild papillary engorgement; slight increase in size

2+ - Obvious increase in size of gingival papilla; hemorrhage on pressure

3+ - Excessive increase in size with spontaneous hemorrhage

4+ - Necrotic papilla

5+ - Atrophy and loss of papilla

Marginal

0 - Normal; no inflammation visible

1+ - Engorgement; slight increase in size; no bleeding

2+ - Obvious engorgement; bleeding upon pressure

3+ - Swollen collar; spontaneous hemorrhage; beginning infiltration to attached gingivae

4+ - Necrotic gingivitis

5+ - Recession of the free marginal gingiva below the CEJ due to inflammatory changes

Attached

0 - Normal; pale rose; stippled

1+ - Slight engorgement with loss of stippling; change in color may or may not present

2+ - Obvious engorgement of attached gingivae with marked increase in redness. Pocket formation present

3+ - Advanced periodontitis; deep pockets evident

At the second appointment, a thorough dental prophylaxis using a rubber cup and flour pumice was performed. Each surface was cleaned for 10 seconds with the cup

revolving at slow speed. The child was then asked to chew a disclosing tablet* to ensure complete removal of the plaque. If stain was observed, the prophylaxis was repeated until there was no visible plaque upon staining. During the entire session, the child was given initial oral hygiene instructions. Following the prophylaxis a Polaroid photograph was taken immediately after staining to substantiate the cleanliness of the teeth. The child was then dismissed from the clinic to return in six hours. He was instructed not to brush his teeth except while at the clinic during the course of the study. No attempt was made to influence the child's diet in any manner.

After six hours, the child was instructed to rinse his mouth with water to dislodge any material alba that might be present. He then chewed a disclosing tablet for two minutes. A second photograph was taken which represented the six-hour accumulation of plaque. Next, the child was told to brush his teeth under supervision with a non-fluoridated tooth paste. The child was not dismissed from the clinic until complete removal of all plaque present was ensured by using disclosing tablets. The child was then instructed to return after 24, 48, and 72 hour periods, at which times the above procedure was repeated. It should be stressed that each observation

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*-Butler Red Coat

period began with a clinically clean tooth or zero plaque accumulation.

Method of Measurement

Using the photographs taken following the dental prophylaxis at the first visit, a tracing with acetate tracing paper was made of the clinical crowns of the teeth to be evaluated. The tracing was then shaded in and transferred to the television area scanner, where the area of the clinical crowns was measured. The area thus derived was the figure used for comparison with the amount of plaque formation. The same procedure was followed to determine the amount of plaque from the subsequent photographs. The area involved was expressed as the percentage of the total area of the clinical crown covered by plaque. With this method of measurement, the progressive formation of plaque could be recorded.

Determination of Television Area Measurement Variability

Ten children in the study were used to determine the variability that may be inherent in this method of intraoral photography and television measurement. The photographs taken at the 6, 24, 48, and 96 hour observation periods were traced to include both the maxillary and mandibular incisors. This provided an

opportunity to evaluate the positioning technique in photographing and the tracing technique at four different intervals. The tracings were then measured to establish the area of the clinical crowns. The measurements were performed at three different times, with the television scanner being reset each time for an objective appraisal of the consistency of the method of measurement. By comparing the individual measurements of the same object, a level of accuracy of this method can be established.

DESCRIPTION OF THE TELEVISION AREA

MEASUREMENT INSTRUMENTATION

FIGURE 1

Description of the Television Area Measurement Instrumentation

The blackened tracing indicating tooth or plaque, as the situation dictated, was back illuminated and viewed by a television camera. The television camera image is electrically inverted, such that the tracing becomes white and the background black. The video image is connected to a small display monitor, the video mixer and the Area Measurement Unit. The purpose of the Measurement Window Generator is to generate two vertical lines and two horizontal lines which may be positioned as desired to restrict the measurement area.

The Area Measurement Unit consists of an internal clock generator, logic control circuitry and output drivers which generates a serial train of output pulses (dots) when the tracing video image enables the unit. This unit is so timed with the television system that the tracing video image is measured once each second and the output pulses (dots) are counted by the Count Display Unit. The count is digitally displayed for one second then zeroed, and the count cycle repeated. The output pulses are also connected to the Video Mixer where they are mixed into the composite image viewed on the Master Monitor. The Area Measurement Unit video splicing level is set to a point just below the spill over of dots occurs and such that the tracing area is completely covered with dots, therefore rendering a numerical dot count proportional to the area of tooth or plaque being measured as the situation indicates.

T. V. AREA MEASUREMENT INSTRUMENTATION ARRANGEMENT

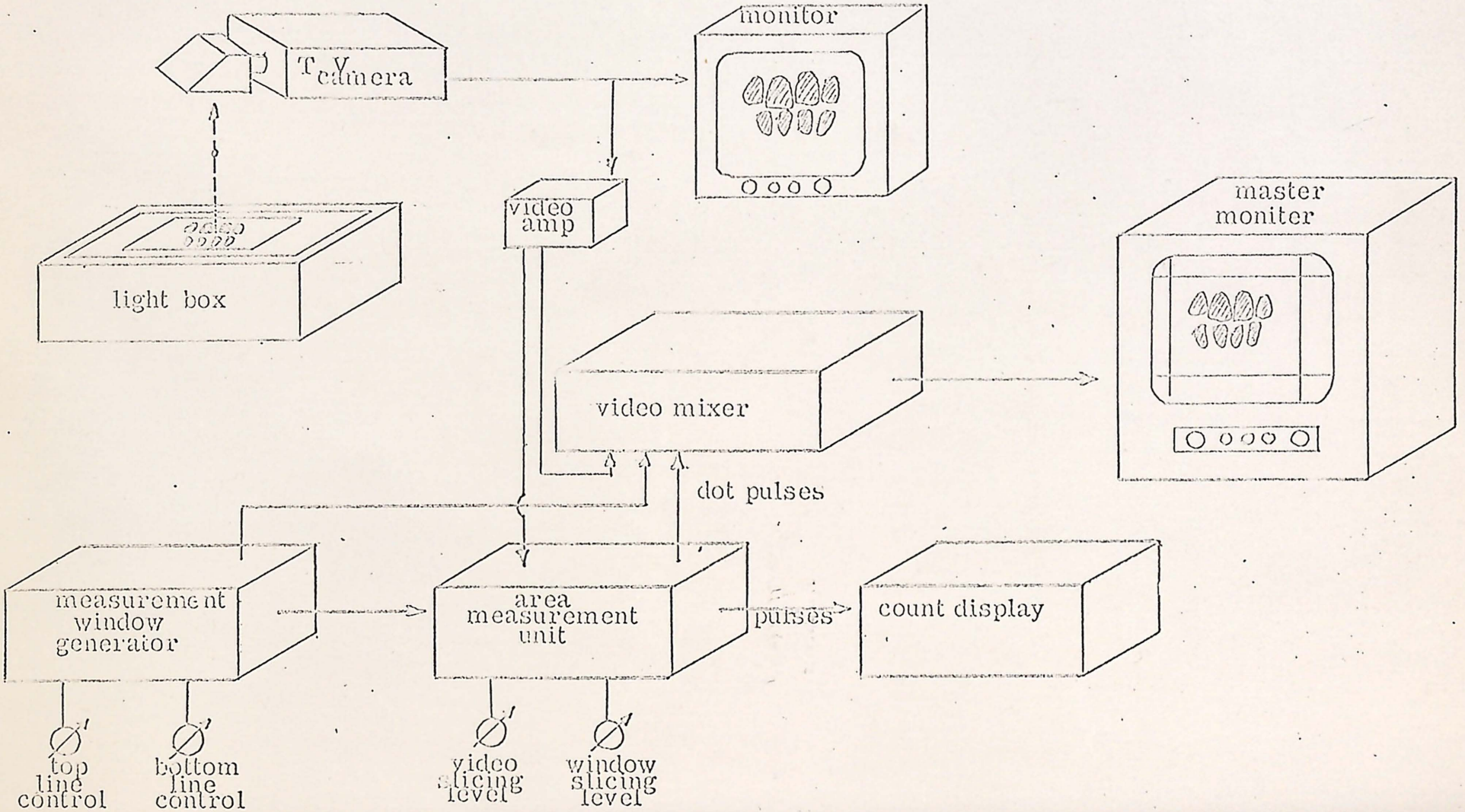

FIGURE 2. The Television Area Measurement Instrumentation Consists of: a. television camera, b. light box, c. monitor, d. video mixer, e. measurement window generator control, f. Area Measurement Unit, g. count display unit, and h. master monitor.

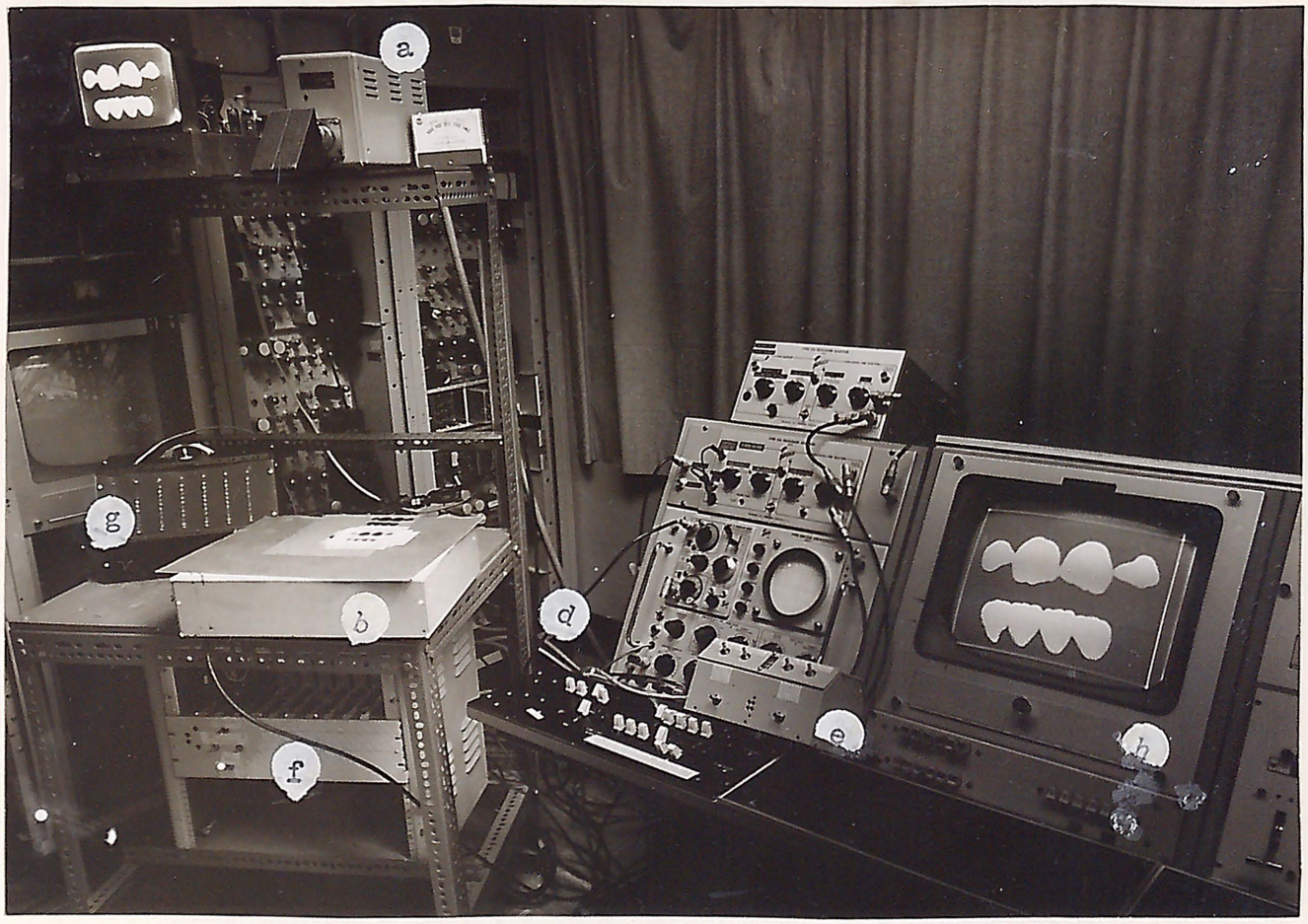
a
g
b
d
f
e
h

FIGURE 3a. The figure, 1804, represents the number of units as assessed by the Television Area Measurement instrument to be the total tooth area for this particular child.

FIGURE 3b. The Television Area Measurement instrument has assessed that 730 units, on 40 per cent plaque coverage has occurred at the 96-hour observation period for the child illustrated above.


1804


0730

RESULTS

The four observation periods for plaque formation of the 56 children in the study represented a total of 224 appointments. Complete data were obtained from 52 children, and of the remaining four each missed one observation appointment. This represents a high degree of cooperation, 98.2 per cent, from the participants in the study.

The range of the DMFS scores for the entire sample was zero to 35. The mean DMFS was 11.84 with a standard deviation of 7.13 (Table 1).

Every child in the group displayed some sign of periodontal disease utilizing Russel's Periodontal Index. The range of scores was .28 to 1.6 representing a mean of .76 with a standard deviation of .27 (Table 1).

The Anterior P-M-A Index examination of the anterior region, which was graded on severity and then the three values added for statistical purposes, revealed a range from zero to forty. Only two children in the group were free of gingivitis in the anterior region, which means that 96.5 per cent of the sample manifested signs of periodontal disease in this area. The mean P-M-A cumulative score was 12.1, with a standard deviation of 8.8 (Table 1).

The absolute amounts of plaque present at the various observation periods expressed as a percentage

of the total tooth surface of surmounted dental and lateral incisors are listed in Table 2.

The mean amount of plaque formed for the entire group at the 6-hour observation period was 1.5 per cent, $\pm$ 2.1 per cent. The children varied from no demonstrable plaque up to 11.2 per cent. The 24-hour observation period revealed a mean amount of plaque of 4.2 per cent, $\pm$ 3.9 per cent, ranging from no demonstrable plaque to 20.6 per cent. A considerable increase was found at the 48-hour period with a mean of 19.4 per cent, $\pm$ 11.7 per cent. The range at this observation period was from 1.7 per cent to 52.3 per cent. The doubling of the time period to the 96-hour observation appointment provided a mean of 39.2 per cent of the tooth surface covered with plaque and a standard deviation of 15.7 per cent. The range for the sample at this period was 8 to 73.1 per cent (Table 2).

The incremental amounts of plaque that were formed between the observation periods were used as the basis for inferences concerning rate of formation, as expressed in Table 3. This is also expressed as a percentage of the total tooth surface that is covered with plaque. For the time period between 6 and 24 hours, the mean amount of plaque formed for the group was 2.7 per cent, with a standard deviation of 3.2 per cent.

The range for the group during this time was from 1.5 per cent to 50.7 per cent. From 48 to 96 hours, the group expressed a mean amount of plaque formation of 19.1 per cent, ranging from 3.1 per cent to 36 per cent with a standard of 10.7 per cent.

The percentages of plaque to total tooth area were calculated. Pearson product moment correlation coefficients were calculated to detect relationships between:

1. DMFS, Russel's Periodontal Index and Anterior P-M-A scores. (Table 4a)
2. Absolute amount of plaque expressed as a percentage of the surface involved with the DMFS, Periodontal Index and Anterior P-M-A scores. (Table 4b)
3. Incremental amount of plaque formed from one observation to the following one with DMFS, Periodontal Index and the Anterior P-M-A scores. (Table 4c)

Results of the Correlation Tests

A very high statistical correlation (r=.811) was found to exist between the Periodontal Index and the Anterior P-M-A scores. However, there was a very low correlation between the DMFS scores and both the Anterior P-M-A (r=.017) and the Periodontal Index

(r=.134). (Table 4a).

There is a statistically significant relationship between the amount of plaque involving the total tooth area at the 48-hour observation period with the Periodontal Index (r=.345*) and with the Anterior P-M-A score, (r=.479**). However, the relationships found at the 6, 24 and 96-hour observation periods were poor. (Table 4b).

In relating the various scores with the incremental amount of plaque formed between observation periods, the amount of plaque formed between 24 to 48 hours was statistically correlated with the Periodontal Index, (r=300*), and the Anterior P-M-A, (R=.445**) (Table 4c).

The correlation between plaque and the Periodontal Index scores, although significant at the five per cent level of confidence is not high in terms of the magnitude of the correlation coefficients. However, the Anterior P-M-A scores are highly correlated with the amount of plaque formed at certain periods and is at the one per cent level of confidence.

A comparison was made between the amount of plaque formed of the maxillary and mandibular central and lateral incisors at the 96-hour observation period.

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* $p < .05$

** $p < .01$

It was found that more plaque formed on the mandibular than the maxillary incisors when expressed as a plaque to tooth ratio, as expressed in Table 5. A T-test for paired observations was utilized for the method of analysis. The t value is 4.564, which is highly significant. The level of confidence for this value is $p < .001$. The mean amount of plaque formed on the mandibular incisors was 46.1 per cent with a standard deviation of 21.6 per cent. The mean for the maxillary incisors was 34.5 per cent with a standard deviation of 15.4 per cent. (Table 5).

Eleven individuals, with four photographs per individual and three television readings of each photograph, were used to estimate the reliability of the method utilizing the television measurement of plaque attempted in this study. The hierarchal, or nested, design in the analysis of variance was used to partition, or separate out, the variation due to individuals, photographs, and television settings. These three sources of variation were then estimated, and reliability was calculated as a percentage of the total variation.

From Table 6, the three different components contributing to the total variation were calculated to be: $\sigma_i^2 = 2282.5$; $\sigma_s^2 = 9.2$ and $\sigma_p^2 = 6.2$.

The σ_i^2 value is expected to be high as this represents the variation in tooth size from one individual to the next. The σ_s^2 value represents the variation that exists in the four different photographs and tracings that were made of each of the eleven children.

If the total variation is the sum of the three components, i.e., $\sigma_i^2 + \sigma_p^2 + \sigma_s^2 = 2297.9$, then the reliability of the television measurement technique may be defined as Reliability = $\frac{\sigma_i^2}{\text{total variation}}$, and the error of the television measurement and photography technique may be defined as Error = $\frac{\sigma_p^2 + \sigma_i^2}{\text{total variation}}$.

Therefore the Reliability = $\frac{2282.5}{2297.9}$ = .993 = 99.3 per cent.

The error inherent in the entire method of measurement utilized in this study is $\frac{9.2 = 6.2}{2282.5}$ = .007 = .7 per cent error.

FIGURES AND TABLES

Case Number	Dmfs	PI	P-M-A Ant.	Case Number	Dmfs	PI	P-M-A Ant.
1	13	.63	8	29	8	.38	5
2	10	.83	10	30	10	.75	10
3	15	.46	6	31	9	.46	9
4	3	.91	21	32	1	.54	7
5	14	.50	3	33	21	.74	15
6	11	.79	18	34	12	1.30	33
7	18	1.08	18	35	12	1.07	13
8	10	.63	6	36	8	.83	9
9	18	1.13	25	37	17	1.08	8
10	9	.52	2	38	17	.65	8
11	12	.62	10	39	2	1.13	40
12	35	.90	9	40	11	.83	14
13	7	1.20	25	41	11	.81	10
14	6	.92	11	42	17	1.56	27
15	12	.42	7	43	4	.83	15
16	8	1.00	24	44	8	.74	7
17	12	.54	1	45	1	.75	18
18	15	.42	1	46	20	.75	6
19	6	.74	10	47	10	.57	17
20	10	.93	13	48	14	.29	0
21	1	.79	15	49	6	.81	11
22	6	.39	0	50	8	.55	9
23	28	.37	4	51	15	.73	10
24	17	.60	3	52	0	.25	0
25	4	.57	5	53	19	.54	15
26	19	.92	15	54	6	.92	14
27	10	.75	8	55	20	.70	10
28	17	.58	12	56	30	1.43	38

TABLE I. THE CARIES AND PERIODONTAL INDICES

Mean DMFS 11.84 Standard deviation 7.13
Mean Periodontal Index .76 Standard deviation .27
Mean Anterior P-M-A total 12.07 Standard deviation 8.78

Case No.	6 Hour	24 Hour	48 Hour	96 Hour	Case No.	6 Hour	24 Hour	48 Hour	96 Hour
1	3.1%	5.0%	10.7%	33.3%	29	.6%	1.1%	23.3%	38.1%
2	2.8	4.5	10.6	44.7	30	.4	.5	12.3	42.3
3	.8	3.6	10.2	21.6	31	1.3	3.2	5.1	25.3
4	1.5	9.0	43.3	73.1	32	.3	15.6	18.7	24.4
5	0	.7	FA*	53.1	33	2.9	5.8	11.2	22.4
6	2.8	5.6	10.6	38.7	34	1.2	4.9	20.7	43.6
7	.8	4.4	23.1	41.3	35	0	6.3	FA*	62.3
8	0	0	1.7	16.9	36	0	1.4	31.2	43.9
9	.6	6.0	39.1	44.4	37	0	3.3	28.0	40.7
10	.4	.7	2.2	27.3	38	.3	4.8	33.1	61.4
11	.4	.5	14.6	47.3	39	0	1.6	52.4	61.1
12	.6	3.8	6.9	27.7	40	.3	.5	3.7	8.1
13	0	1.2	20.4	20.9	41	0	0	3.3	19.2
14	1.4	2.1	10.6	14.9	42	11.2	15.3	16.9	17.1
15	1.8	4.9	30.1	60.1	43	2.9	4.4	26.5	62.5
16	0	6.4	14.9	31.2	44	9.3	11.3	15.2	29.8
17	.7	4.7	19.5	53.0	45	2.1	11.7	FA*	49.7
18	1.4	3.4	6.1	12.2	46	.5	.6	17.0	36.1
19	0	.5	15.0	32.7	47	1.0	1.4	13.3	29.7
20	1.4	2.0	17.7	29.3	48	4.8	6.1	19.7	60.5
21	.2	.2	6.1	42.2	49	1.9	4.5	21.3	39.4
22	1.3	5.0	17.0	47.1	50	6.3	7.6	21.4	39.6
23	.6	3.1	14.0	40.2	51	.2	4.6	36.6	55.7
24	.7	2.0	FA*	22.7	52	2.3	8.5	20.9	24.0
25	0	2.4	9.7	28.2	53	0	.6	24.2	59.1
26	1.7	3.3	41.8	47.8	54	0	.7	27.9	51.9
27	2.7	4.6	20.5	48.3	55	.3	.7	28.7	62.0
28	3.7	4.3	18.9	32.9	56	1.4	7.1	47.1	60.7

TABLE II. PERCENTAGE OF PLAQUE TO TOOTH SURFACE OF THE PERMANENT CENTRAL AND LATERAL INCISORS AT EACH OBSERVATION PERIOD.

6 hour mean 1.48 per cent; standard deviation 2.15
24 hour mean 4.15 per cent; standard deviation 3.90
48 hour mean 19.44 per cent; standard deviation 11.75
96 hour mean 39.17 per cent; standard deviation 15.73

* Failure Appointments

Case No.	0-6 Hours	6-24 Hours	24-48 Hours	48-96 Hours	Case No.	0-6 Hours	6-24 Hours	24-48 Hours	48-96 Hours
1	3.1	1.2	5.6	22.6	29	.6	.6	22.2	14.8
2	2.8	1.7	6.1	34.1	30	.4	.2	11.8	30.0
3	.8	2.8	6.6	11.4	31	1.3	2.0	1.9	20.3
4	.5	7.5	34.3	29.9	32	.3	20.4	1.9	4.4
5	0	.7	FA*	48Hr	33	2.9	2.9	5.3	11.2
6	2.8	2.8	4.9	28.2	34	1.2	3.7	16.0	22.7
7	.8	3.5	18.8	18.1	35	0	6.3	FA*	48Hr
8	0	0	1.7	15.2	36	0	1.4	29.8	12.8
9	.6	5.3	33.1	5.3	37	0	3.3	24.7	12.7
10	.4	.2	1.5	25.2	38	.3	4.5	28.3	28.3
11	.4	.1	14.1	32.7	39		1.6	50.8	8.7
12	.6	3.1	3.1	20.8	40	.3	1.2	3.2	4.3
13	0	1.2	19.2	6.5	41	0	0	3.3	15.9
14	1.4	.7	8.5	44.3	42	11.2	4.1	2.3	44.1
15	1.8	3.1	25.2	30.1	43	2.9	1.5	22.1	36.0
16	0	6.4	8.5	16.3	44	9.3	2.0	4.0	14.6
17	.7	4.0	14.8	33.6	45	2.1	9.7	FA*	48Hr
18	1.4	2.0	2.7	6.1	46	.5	.2	16.3	19.0
19	0	.5	14.6	17.7	47	1.0	.7	12.9	16.4
20	1.4	.7	15.7	11.6	48	4.8	1.4	13.6	40.8
21	.2	0	5.9	36.1	49	1.9	2.6	16.8	18.1
22	1.3	3.9	11.8	30.1	50	6.3	1.3	13.8	18.2
23	.6	2.4	11.0	26.2	51	.2	4.4	32.1	19.1
24	.7	1.3	FA*	48Hr	52	2.3	6.2	12.4	3.1
25	0	2.4	7.3	18.6	53	0	.6	23.6	34.9
26	1.7	1.7	38.5	6.0	54	0	7	27.2	24.0
27	2.7	2.0	15.9	27.8	55	.3	.3	3.8	33.3
28	3.7	.6	14.6	14.0	56	1.4	5.7	40.0	13.6

TABLE III. INCREMENTAL PERCENTAGE OF PLAQUE RELATIVE TO TOOTH SURFACE OF THE PERMANENT CENTRAL AND LATERAL INCISORS FORMED BETWEEN OBSERVATION PERIODS.

0 to 6 hour mean 1.48 per cent; standard deviation 2.15
6 to 24 hour mean 2.7 per cent; standard deviation 3.23
24 to 48 hour mean 15.37 per cent; standard deviation 11.78
48 to 96 hour mean 19.12 per cent; standard deviation 10.68

* Failure Appointments

	DMFS SCORE	PERIODONTAL INDEX
ANTERIOR P-M-A INDEX	.017	.811**
PERIODONTAL INDEX	.134	---

TABLE IVa. Correlation coefficients, r's, found between DMFS scores, Periodontal Index and the Anterior P-M-A Index.

	DMFS SCORE	PERIODONTAL INDEX	ANTERIOR P-M-A INDEX
6 hour	.007	.124	.057
24 hour	-.125	.156	.143
48 hour	.071	.345*	.479**
96 hour	.053	.062	.184

TABLE IVb. Correlation coefficients, r's, found at the 6-, 24-, 48-, and 96-hour observation periods with the DMFS scores, Periodontal Index and the Anterior P-M-A Index.

	DMFS SCORE	PERIODONTAL INDEX	ANTERIOR P-M-A INDEX
24-6 hour	-.155	.106	.135
48-24 hour	.092	.300*	.445**
96-48 hour	.047	-.323	-.259

TABLE IVc. Correlation coefficients, r's, found between the amount of plaque formed from one observation period to the next and the DMFS scores, Periodontal Index and the Anterior P-M-A Index.

* denotes $P < .05$
** denotes $P < .01$

Case No.	Mandibular Incisors	Maxillary Incisors	Case No.	Mandibular Incisors	Maxillary Incisors
1	24.3	40.5	29	51.4	29.2
2	78.4	20.9	30	39.1	44.1
3	23.1	20.6	31	29.5	22.7
4	92.0	61.9	32	14.1	14.6
5	66.7	43.7	33	37.5	11.3
6	74.1	17.0	34	59.7	33.6
7	60.0	27.4	35	87.7	48.0
8	16.0	17.6	36	66.7	29.9
9	61.4	34.0	37	31.0	46.7
10	27.8	25.9	38	69.8	54.9
11	44.9	50.5	39	80.4	45.7
12	27.9	27.5	40	1.3	12.9
13	18.3	22.8	41	30.8	10.5
14	12.3	16.7	42	21.6	13.5
15	57.7	62.0	43	55.6	67.1
16	18.3	40.7	44	51.9	17.5
17	58.5	48.8	45	59.0	42.9
18	34.5	17.8	46	41.9	31.8
19	34.3	31.4	47	48.1	17.1
20	24.0	32.0	48	68.3	55.2
21	61.7	28.7	49	28.1	47.3
22	39.3	52.2	50	49.2	32.9
23	56.1	29.6	51	76.5	42.5
24	29.7	17.4	52	28.9	20.1
25	38.5	20.8	53	65.3	55.4
26	67.6	35.1	54	62.2	44.1
27	46.4	49.5	55	52.8	67.0
28	44.6	25.3	56	68.9	53.8

TABLE V. Percentage of plaque relative to tooth surface of the permanent maxillary and mandibular central and lateral incisors at the 96 have observation period.
Mean for mandibular incisors 46.1 percent; standard deviation 21.6.
Mean for maxillary incisors 34.5 percent; standard deviation 15.4.

Sources of Variation	Degrees of Freedom	Mean Square	Mean Square Estimates*
Between Individuals	10	27417.2	$\sigma_s^2 - 3\sigma_p^2 - 12\sigma_i^2$
Between Photographs within Individuals	33	27.8	$\sigma_s^2 - 3\sigma_p^2$
Between Settings within Individuals	88	9.2	σ_s^2
TOTAL	131		

TABLE VI. The Hierarchal Analysis of Variance Table: The method of analysis utilized to calculate the reliability of the method of measurement.

σ_i^2 variance attributable to differences between individuals

σ_p^2 variance attributable to differences between photographs

σ_s^2 variance attributable to differences between TV settings

Figure 4a. Graph depicting the rate of plaque formation utilizing the mean absolute percentages observed during this study.

Figure 4b. Graph depicting the rate of plaque formation utilizing the mean incremental percentages observed during this study.

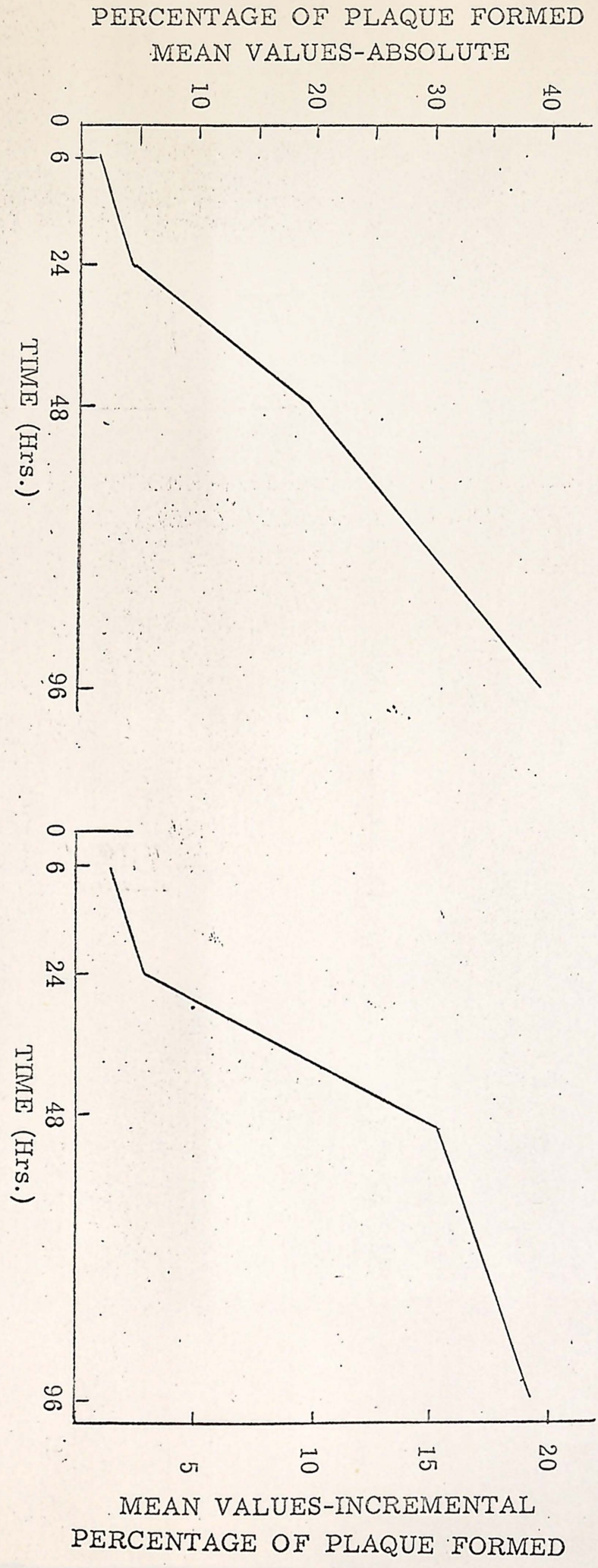
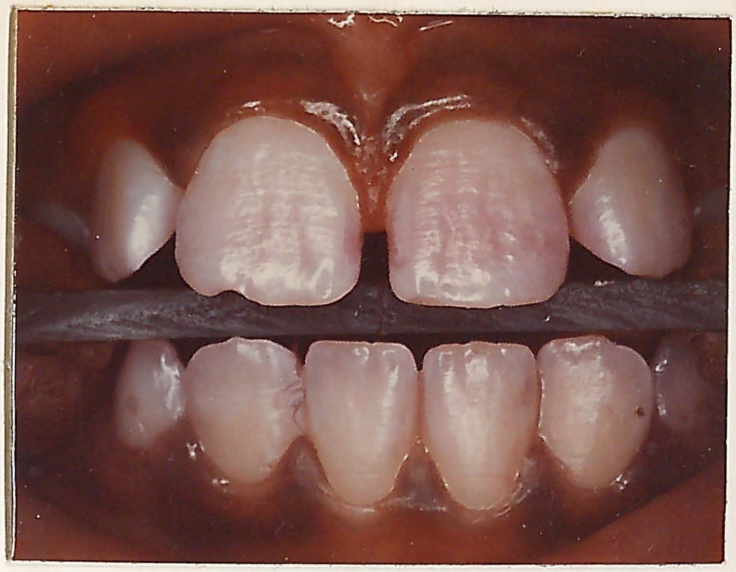

PERCENTAGE OF PLAQUE FORMED
MEAN VALUES-ABSOLUTE
10
20
30
40
0
6
24
48
96
TIME (Hrs.)
0
6
24
48
96
TIME (Hrs.)
5
10
15
20
MEAN VALUES-INCREMENTAL
PERCENTAGE OF PLAQUE FORMED

Figure 5a. Photograph demonstrating .7 per cent plaque present at the 6-hour observation period of case number 9.

Figure 5b. Photograph demonstration 6.00 per cent plaque present at the 24-hour observation period of case number 9.



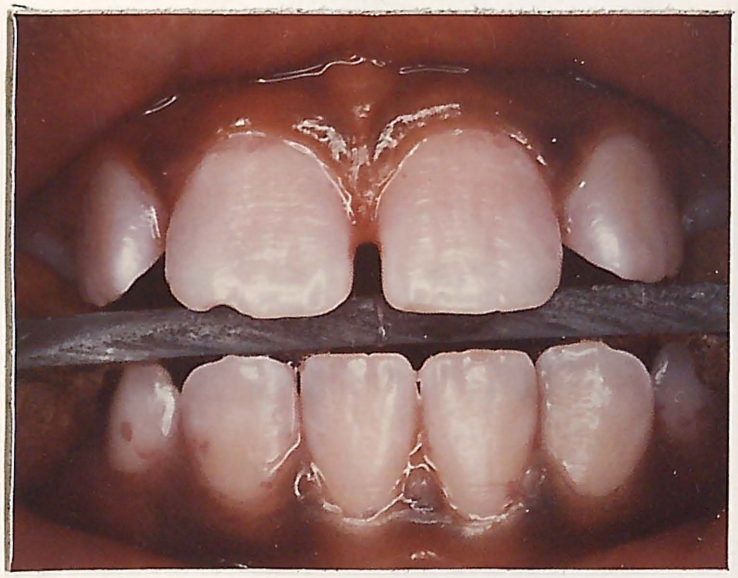


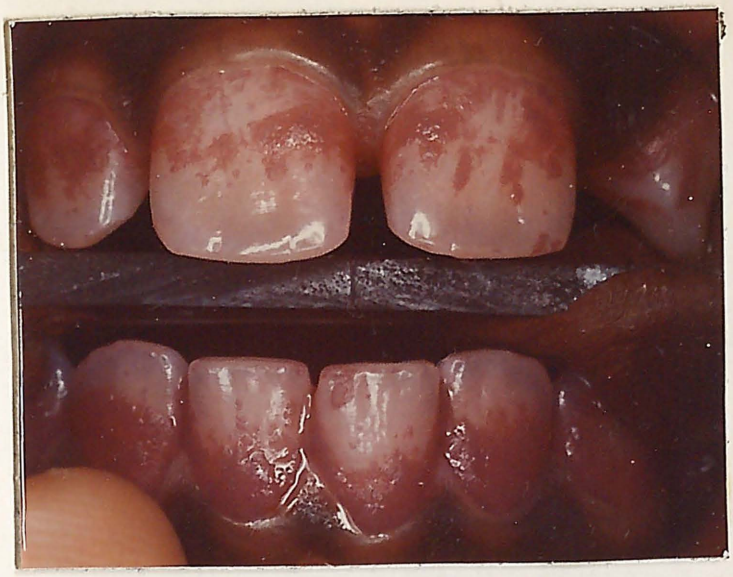
Figure 5c. Photograph demonstrating 39.10 per cent plaque present at the 48-hour observation period of case number 9.

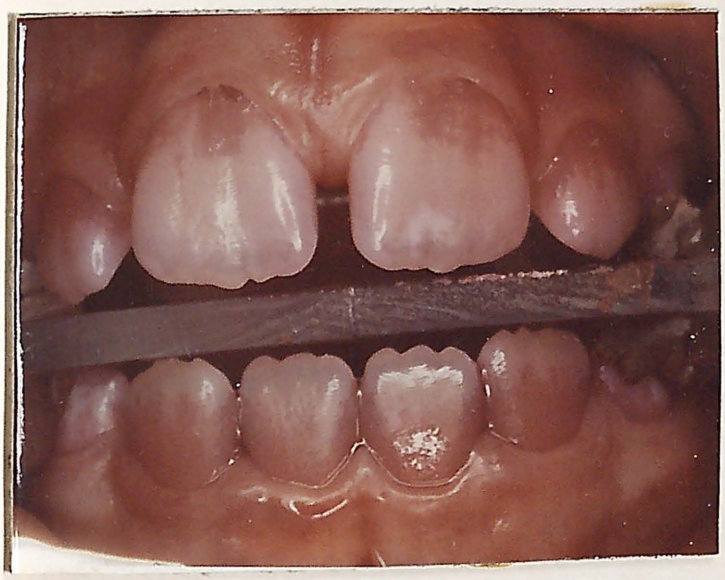
Figure 5d. Photograph demonstrating 44.40 per cent plaque present at the 48-hour observation period of case number 9.

Figure 6a. Case No. 48 Photograph utilized in the study of the plaque present at the 96-hour observation period. 60.54 per cent

Figure 6b. Case No. 52 Photograph utilized in the study of the plaque present at the 96-hour period. 24.03 per cent

Figure 6c. Case No. 36 Photograph utilized in the study of the plaque present at the 96-hour observation period. 43.97 per cent





DISCUSSION

In formulating the protocol for this study, the immediate concern was how to motivate 56 children and their parents to participate in a study which required so many observation periods in a relatively short time. It is felt that the resulting participation of 98.2 per cent can be directly attributed to the administration of concentrated oral hygiene instructions to the children by the hygienists at each observation period. This result demonstrates the interest of the parents of these children in having them receive this type of information.

According to the Anterior P-M-A Index for the presence and severity of gingivitis, 96.5 per cent of the sample manifested signs of periodontal disease, which was almost identical to that reported by Moore.[139]

The validity of using only the Anterior P-M-A Index to gain a reflection of gingivitis of the entire mouth has been substantiated by McDonnel and Domalkas[140] and others.[141] Moore[139] found a correlation of r = .91 between the Total P-M-A Index and the Anterior P-M-A Index.

The use of the Russel's Periodontal Index in this age group presented some problems and many decisions had to be made during the examination period. Children

8 through 12 represent a group in which the dentition is classically undergoing transition. As a result, there were many teeth which were either undergoing eruption or exfoliation with its expected disruption of the surrounding tissue. These teeth were eliminated from the score. The findings of 100 per cent involvement of periodontal disease in the sample and a mean score of .76 for this age group are high when compared with the findings of more experienced examiners studying a similar group.[142] Many epidemiologists feel that those examiners with limited experience and who have not been calibrated tend to be overly critical and score high. It is suggested that this may be the reason for high score in this sample.

The correlation coefficient of r = .811 between the Anterior P-M-A Index and Russel's Periodontal Index is very interesting, especially when large samples are being studied and time and personnel are limited. The Anterior P-M-A Index required far less time for the examination than did the Periodontal Index and the author felt that it was an easier index to use for this age group.

Numerous investigators have attempted to determine a relationship between periodontal disease and caries.[143,144] Both positive[145,146] and negative[144,147,148] correlations

have been presented. The correlation found in this study between the DMFS scores and the Anterior P-M-A Index (r=.017) and the Periodontal Index (r=.134) can hardly be considered significant. In view of the fact that caries and periodontal disease have a common etiology factor, i.e. plaque, whether or not a common factor, the lack of relationship between the two diseases is, indeed, difficult to explain.

In evaluating the mean absolute amounts of plaque formed for the entire group at the four observation periods, it was noted that the percentage of the area of plaque covering the tooth surface involved approximately doubled from the 6 to the 24-hour period and from the 48- to the 96-hour period. However, the percentage of the surface involved increased almost five times from the 24- to the 48-hour observation period. Figure 4 demonstrates this pattern very well. This clinical observation reflects the findings of such investigators as McDougall,[24] Bjorn and Carlson,[93] and Ritz.[76] They attributed this increase in formation after 24-hours to a more favorable oxidation-reaction potential present in the older plaque which enhanced the metabolism of anaerobic organisms.

It is interesting that 77 per cent of this sample manifested some measurable amount of plaque just 6 hours

after complete plaque removal. This was an unexpected finding. Those children with large amounts of plaque formed at the 6-hour observation period did not necessarily have the larger amounts observed at the 96-hour observation period.

The findings of this study revealed that in 96-hours following a dental prophylaxis from 8 to 73 per cent of the tooth surface observed was covered with plaque, with a mean of 39.2 per cent for the entire group. The mean was approximately three times the amount reported as occurring in adults for observation periods of 7 days by Arnim[91] and Kinoshita.[99] The possibility of the disclosing tablets inhibiting the accumulative growth of the plaque, as suggested by Bjorn and Carlson,[93] may account for some of the differences in the studies compared. It is apparent that children in this age group can form larger amounts of plaque in shorter periods of time than adults.

Positive statistical correlations were found when comparing the amount of plaque formed at the 48-hour observation period with the Periodontal Index ($r = .345$), and the Anterior P-M-A ($r = .479$). Similar correlations were also found in evaluating the incremental amounts of plaque formed. This is the period in which the plaque undergoes a rapid growth and increases

in thickness. Its pathologic potential may also increase at this time. However, the author cannot explain why the correlation is not also apparent at the 96-hour observation period.

Arnim[91] reported that there seemed to be more plaque on the lower teeth than the upper. Silness and Loe,[134] on the other hand, observed no major differences in the tendency for plaque formation between the maxilla and the mandible. The children in this study displayed a very distinct difference in that, when expressed as a percentage of the area involved, the mandibular teeth had more plaque than the maxillary teeth. The mean amount of plaque formed on the mandibular incisors was 12 per cent higher than for the mean amount recorded for the maxillary incisors at a highly significant level.

Kaminsky and Kleinberg[100] found that the mandibular teeth are in contact with a greater amount of saliva due to both proximity to salivary glands and to the effects of gravity. Because of the proximity to sub-maxillary saliva, there was less ash in mandibular plaque, to which they feel contributes to higher pH levels of the area. Perhaps, it is the difference in pH levels that enhances increased plaque formation in the mandibular incisor region.

In evaluating plaque formation in children, it is readily apparent that the amount, rate, and appearance of the plaque varied greatly from one child to another as shown in Figure 6.

The author feels that a factor in accelerated plaque formation in many children was the presence of many defects in the enamel surface. This is analagous to the findings of Brebau and Muhleman[87] on the increase in plaque found with roughened mylar strips adapted to the teeth to collect plaque. The inability to remove all the bacteria from these defects by the dental prophylaxis would also be a factor in the reformation of the plaque.

A variable which the design of this study made no attempt to control was the diet of each child. It is reasonable to assume that the amount of carbohydrates consumed varied greatly from child to child. The varying amounts of fibrous foods present in the diets were not a cause for concern.[91]

The utilization of a technique which provides as little as .7 per cent error is certainly encouraging. However, the verification of the measurements could have been even more conclusive. It was difficult to distinguish between errors that may have occurred either in tracing the photographs or in making the

television setting than was found in the technique for the photographs. This was attributed to the daily fluctuation of voltage provided to the equipment. In future studies utilizing this equipment, the author suggests that several readings be taken for each area to be measured and average readings utilized. Also, when comparative measurements are to be made within the same object or individual, that all the measurements for that particular sample should be made on the same day.

In evaluating the overall technique, it is felt that the method was more accurate and far less time-consuming and cumbersome than those previously reported methods of other investigators attempting a similiar project. The use of the Polaroid CU5 camera and oriented compound bites provided excellent and consistent photographs. By this method, an acceptable photograph was insured at each observation period before the child was dismissed. After the tracings were made, which proved to be the most time-consuming aspect of the study, the actual measuring of the areas was exceedingly fast and efficient.

SUMMARY AND CONCLUSIONS

Plaque formation at 6-, 24-, 28- and 96-hour intervals was observed in 56 children aged 8 through 12 years to establish basic information regarding its rapidity of formation after initial removal. The rate of formation was recorded by utilizing oriented Polaroid color photographs at a consistent three times magnification and was measured by televisions measurement instrumentation. Also, examinations for caries and periodontal disease were conducted on each child, and this information correlated with the amount of plaque recorded at each observation period.

Correlations were also computed between the Anterior P-M-A Index and the Periodontal Index.

1. In comparing the amount of plaque formed by these children with results reported from similar studies conducted on adults, it is apparent that children in this age group form more plaque in less time than adults. Seventy-seven per cent of the sample displayed measurable amounts of plaque just six hours after a thorough dental prophylaxis.

2. No correlation was found to exist between the amount of plaque formed at the various observation periods and the DMFS scores.
3. A significant correlation was found between the amount of plaque present at the 48-hour observation period and the Anterior P-M-A and the Periodontal Index of the children in the sample.
4. A high correlation, r = .811 was found between the Anterior P-M-A and the Periodontal Index. The Anterior P-M-A was considered to be the more desirable index for studying the periodontal status of a large number of children in this age group.
5. No correlations were found between the periodontal indices and the DMFS scores.
6. The mandibular incisors were found to form more plaque than the maxillary incisors at a highly significant level when the plaque is expressed as a percentage of the tooth surface that it covers.

7. The reliability of color Polaroid photographs, acetate tracing and the television area measurement instrumentation was calculated to be 99.3 per cent. The entire method was felt to be easier and less cumbersome than those reported in the literature to provide the same information.

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ABSTRACT

The purpose of this study was to determine the rate of plaque formation in children and its relationship to their periodontal and caries indices and to evaluate a television area measurement instrument. Plaque formation at 6-, 24-, 48-, and 96-hour intervals was observed in 56 children aged 8 through 12 to establish the rapidity of its reformation after a thorough dental prophylaxis. The plaque was recorded with disclosing agents and oriented Poloroid photographs and measured by newly developed television and electronic area measurement instrumentation which proved to be highly reliable. The presence of caries and periodontal disease was then correlated with the amount of plaque recorded at each observation period. A high correlation was found between the Anterior P-M-A and the Periodontal Index. No significant correlations were found between the periodontal indices and the DMFs scores or with the amount of plaque present and the DMFs scores. Seventy-seven per cent of the children displayed measurable amounts of plaque just 6 hours after a thorough dental prophylaxis. In comparing the amount of plaque formed by these children with results reported from similar studies conducted on adults, it is apparent that children in this age group form more plaque in less time than adults. A significant correlation was found between the amount of plaque present and the periodontal indices at the 48-hour examination.